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American Dairy Science Association *Meets on Pacific Coast in July*

The members of the American Dairy Science Association at the Ames meeting last Summer voted to hold the next Summer meeting on the Pacific Coast. An elaborate itinerary of tours has been arranged, which will give an unusual opportunity to those who attend.

The formal program will be held at the University of California, Berkeley and Davis, July 13, 14, 15, 1931. Notices have been sent out to the heads of divisions in the various colleges and to commercial organizations, in which requests have been made for suggestions for the program.

The pre-convention tour will start at Los Angeles on July 7 and will include Los Angeles and vicinity; Yosemite Valley; visits to Dairy Farms and Dairy Plants; Stanford University and San Francisco.

The post-convention tour will include a three-day trip over the Redwood Highway from San Francisco to Eureka and Grant's Pass, Oregon, covering a distance of 450 miles through primitive Redwood Forests and along the border of the Pacific Ocean. Automobile transportation will be furnished for both of these tours.

From Grant's Pass, Oregon, to Portland, trips have been arranged to the State Agricultural College, Corvallis; to the dairy sections along the Coast, Oregon Jersey Cattle Club Show; Tillamook Cheese Plants; Lower Columbia Dairy Association at Astoria; Mount Hood and then north to Seattle, Washington, and returning to the East by one of the several possible routes, passing through several States of the Northwest with opportunities to visit the State Universities and State Agricultural Colleges and scenic wonders.

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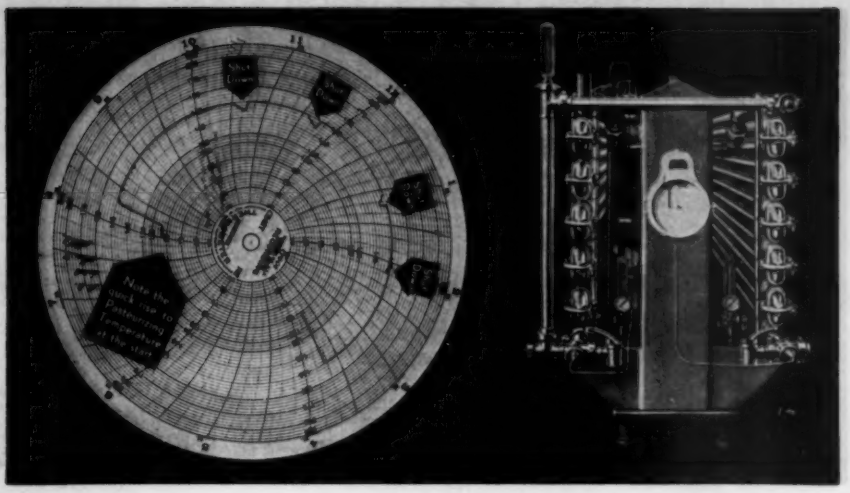

A typical thermal recorder chart (actual) from a Cherry-Burrell "Isotherm" Milk Heater (right). Note the quick, positive rise to pasteurizing temperature at the start, the evenness with which this temperature is maintained.

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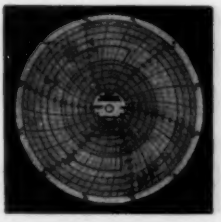

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A TEST FOR THE DETECTION OF MILK UNSTABLE TO HEAT*

GUY A. RAMSDELL, WM. T. JOHNSON, JR., AND FRED R. EVANS

Bureau of Dairy Industry, U. S. Department of Agriculture

INTRODUCTION

An accurate and rapid method for the detection of milk unstable to heat should be of value to the evaporated milk industry. Unfortunately, the literature has failed as yet to reveal such a test. Although a great deal of work has been done in ascertaining the relationships between heat stability and titratable acidity (5), true acidity expressed in pH values (5) (3), concentration of mineral salts (5) (3), and reaction towards alcohol of the whole milk samples (4) (2) (1), not any of these comparisons have been found to be sufficiently definite for advantageous use in grading milk for sterilization purposes. In fact, no test is known wherein the reactions of the test duplicate the reactions produced when milk is heated to sterilization temperatures and, consequently, serve as a criterion of its heat stability. However, a test that will give a truer analysis of the fitness of milk for condensing than any of the existing methods should be of value, and such a test is described in this paper.

The test was developed from the results obtained when making comparisons between heat stability values of the concentrated products and the precipitating actions of numerous acids and salts on the whole milk samples, when such mixtures were heated to a temperature of boiling water. Mono basic potassium phosphate was finally selected, since it was found to be the most satisfactory precipitating agent. The actual coagulation was brought about through the combined action of the added acid salt and heat.

This paper records a description of the test, results obtained, and a discussion of its limitations. Data are also presented show-

* Received for publication June 19, 1930.

ing the relationship between heat stability and minimum concentration of phosphate solution necessary to produce initial coagulation of the milk when added to milk in the manner described in the test.

EXPERIMENTAL

The milk was obtained from herds representing Holstein, Jersey, and Guernsey stock. However, some samples were from registered cattle all of one breed. The samples were collected during the spring, fall, and winter months.

The heat stabilities were determined by forewarming the milk to 95°C. for 10 minutes, concentrating in a partial vacuum, standardizing to 18 per cent solids not fat, sealing in tin cans, and finally sterilizing in a pilot sterilizer. Fifteen minutes were taken to bring the milk to the sterilization temperature of 121°C. Whole milk was always used so that the concentrated product contained approximately 6 to 12 per cent fat. Small changes in fat content had no appreciable effect on the phosphate test.

Throughout this work, the phosphate test was conducted in the following manner:

Solution used: 0.5 M KH_2PO_4 (68.1 grams of mono basic potassium phosphate were dissolved in water and the solution diluted to one liter).

Two cubic centimeter samples of milk were transferred to Pyrex test tubes of approximately 20 cc. capacity, and 0.20 cc. of the phosphate solution added accurately to each tube by means of a 1 cc. pipette, calibrated in 0.1 cc. divisions. The pipette was of small bore, so that the divisions were far enough apart to permit measurements within an accuracy of 0.01 cc.

The contents of the tubes were then thoroughly mixed and the tubes immersed in boiling water for 5 minutes. They were then removed, cooled, and the mixture examined for the presence of a curd. Any coagulation indicated that the concentrated product was of low heat stability.

The test could be made applicable to factory routine if larger amounts of both milk and phosphate solution were used. A satisfactory proportion would be 10 cc. of milk and 1 cc. of the phos-

phate solution. This would permit the use of a burette for dispensing the phosphate solution. Likewise, the milk could be measured out very rapidly by means of a 10 cc. dipper. With reasonable caution, 10 cc. plus or minus 0.1 cc. of milk can be obtained by this means.

The use of these larger amounts of milk and phosphate solution would obviously reduce errors in measuring, and since there is a fairly definite relationship between the amount of milk and con-

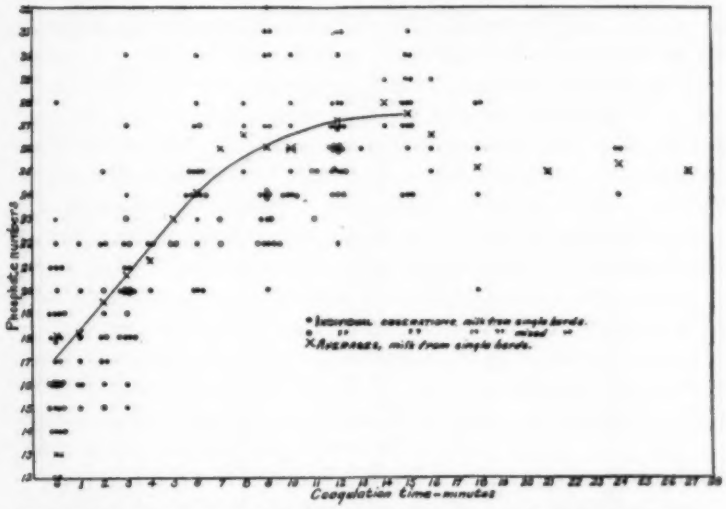

FIG. 1. RELATION BETWEEN PHOSPHATE NUMBERS AND COAGULATION TIME

centration of phosphate solution to produce a curd, results obtained by both methods on the same sample of milk should be comparable. However, when using these larger amounts, care must be taken to have the mixture immersed below the top of the boiling water to insure the necessary heating.

A series of comparisons were first made between the actual concentration of phosphate solution necessary to produce initial coagulation of whole milk samples and the heat stability of the respective evaporated products. The object of this inquiry was

to determine the amount of solution to use in the test. The procedure was similar to that described in the phosphate test, only, in addition, increasing amounts of the solution were added to 2 cc. portions of each sample until the amount within 0.02 cc. was found that would just produce initial coagulation. Approxi-

TABLE 1

*Comparison between coagulation time and average phosphate numbers of milks of similar heat stability values**

COAGULATION TIME	PHOSPHATE NUMBER (AVERAGE)	NUMBER OF TESTS
minutes		
0	18.1	25
1	18.2	6
2	19.5	8
3	20.7	16
4	21.3	3
5	23.0	1
6	24.1	16
7	26.0	1
8	26.6	3
9	26.0	17
10	26.0	8
12	27.2	17
13	26.0	1
14	28.0	2
15	27.5	14
16	26.6	3
18	25.2	5
21	25.0	1
24	25.3	3
27	25.0	1
30	28.0	1
33	27.0	1
36	28.0	1

* Single herd milk samples.

mately 150 individual comparisons were made and the results are plotted in figure 1. A summary of these observations is shown in table 1. The number of fractional cubic centimeters necessary to curdle the milk, multiplied by 100, is designated as the phosphate number. These values are listed in the second column of this table.

The heat stabilities of the milks varied from 0 to 36 minutes and the phosphate numbers from 12 to 32, which represents a considerable range of milks both as to heat stability and phosphate resistance.

As is observed upon inspection of figure 1, there is a striking agreement in the heat stability of those milks that have a phosphate number of 20 or less; over 86 per cent of them coagulated in the sterilizer in less than 4 minutes. They were milks of distinctly poor heat resistance. This relationship is even more pronounced in composite samples from mixed herds. All of the composite samples that had a phosphate number of 20 or less coagulated before 4 minutes. With increasing phosphate numbers there is at first a general trend towards an increase in heat stability values. However, this relationship does not exist in milks of high phosphate numbers. A high phosphate number may not necessarily indicate a high heat resistant milk. In fact, samples with a phosphate number of 24 have coagulated in anywhere from 3 to 24 minutes under sterilization, while one sample with a phosphate number of 32 coagulated in only 9 minutes.

The relationship between coagulation time and average phosphate numbers of milks of the same heat resistance (listed in table 1) is also plotted in figure 1. A fairly definite curve can be traced up to a limiting value of 12 minutes in coagulation time. Beyond this there is little or no correlation.

Table 2 shows the results obtained when applying the phosphate test to cull out the unstable milks. The procedure was to separate from 20 to 120 samples representing as many herds selected each day into three composite batches, A, B, and C. Composite A consisted of an aliquot of the samples that coagulated with the phosphates test, composite B an aliquot of the samples that did not coagulate with the phosphate test, and composite C an aliquot of all the sample selected each day.

The phosphate numbers of these composites were then determined, as well as the heat stability of their forewarmed and concentrated products. The stabilities of the A milks were in every run less than the C milks, and no batch of A milks held up in the sterilizer longer than 3 minutes, while approximately 70 per cent

TABLE 2

Stability of composite samples of milk separated on basis of phosphate test

NUMBER	COMPOSITE	COMPOSITION OF COMPOSITES		COAGULATION TIME	CHANGE IN STABILITY FROM TOTAL COMPOSITE (C)
		A	B		
		per cent	*per cent*	*minutes*	*minutes*
1	A*	100	0	0	5−†
	B	0	100	12	7+
	C	32	68	5	
2	A	100	0	0	5−
	B	0	100	6	1+
	C	21	79	5	
3	A	100	0	1	6−
	B	0	100	9	2+
	C	33	66	7	
4	A	100	0	0	9−
	B	0	100	12	3+
	C	15	85	9	
5	A	100	0	0	9−
	B	0	100	12	3+
	C	27	73	9	
6	A	100	0	3	9−
	B	0	100	15	3+
	C	27	73	12	
7	A	100	0	0	10−
	B	0	100	12	2+
	C	20	80	10	
8	A	100	0	0	2−
	B	0	100	14	12+
	C	24	76	2	
9	A	100	0	0	9−
	B	0	100	12	3+
	C	26	74	9	
10	A	100	0	0	9−
	B	0	100	12	3+
	C	20	80	9	
11	A	100	0	0	1−
	B	0	100	5	4+
	C	36	64	1	

TABLE 2—*concluded*

NUMBER	COMPOSITE	COMPOSITION OF COMPOSITES		COAGULATION TIME	CHANGE IN STABILITY FROM TOTAL COMPOSITE (C)
		A	B		
		per cent	*per cent*	*minutes*	*minutes*
12	A	100	0	3	6−
	B	0	100	12	3+
	C	34	66	9	
13	A	100	0	0	9−
	B	0	100	12	3+
	C	27	73	9	
14	A	100	0	0	9−
	B	0	100	12	3+
	C	40	60	9	
15	A	100	0	0	3−
	B	0	100	6	3+
	C	27	63	3	
16	A	100	0	0	7−
	B	0	100	11	4+
	C	17	83	7	
17	A	100	0	2	9−
	B	0	100	11	0
	C	25	75	11	
18	A	100	0	3	9−
	B	0	100	12	0
	C	16	84	12	
19	A	100	0	2	8−
	B	0	100	10	0
	C	45	55	10	

* A Composite of milks that coagulated with the phosphate test.
B Composite of milks that did not coagulate with the phosphate test.
C Composite of all milks selected each day.
† − Decrease in stability.
\+ Increase in stability.

coagulated at 0 minutes, or before the sterilization temperature was attained. It is observed that the composites made up of samples that coagulated with the phosphate test were invariably

poor heat resistant milks. Hence these results afford excellent evidence of the value of the phosphate test to detect milks of this class. The increase in stability of the B milks over that of the A milks ranged from 5 to 14 minutes. In runs Nos. 17, 18, and 19, no improvement was noted in B milks over that of the total batch or C milks. However, the stability of B milks has never been less than the C milks.

These observations are shown in figure 2. The area above the C line represents an increase in stability over C milks, and the area below a decrease in stability.

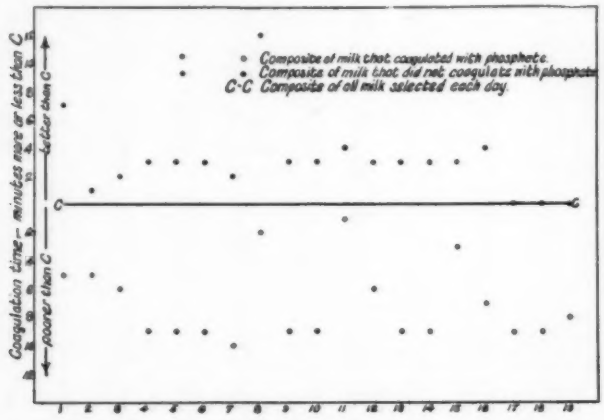
Composite of milk that coagulated with phosphate.
Composite of milk that did not coagulate with phosphate.
C-C Composite of all milk selected each day.
Coagulation time – minutes more or less than C
better than C
poorer than C
C

FIG. 2. COMPARATIVE STABILITY OF COMPOSITE SAMPLES OF MILK SEPARATED ON THE BASIS OF REACTION TO THE PHOSPHATE TEST

The composites where no improvement was made by separating the unstable milks (A milks) gave nearly similar phosphate numbers to that of the C milks, with the exception of sample 19, where the phosphate number of batch C was 24 and batch B, 29.

A comparison between pH values and phosphate numbers failed to show any consistent relationship. Samples with similar phosphate numbers have had pH values anywhere from 6.40 to 6.70. Only in those samples where the H-ion concentration is distinctly abnormal, no doubt due to bacterial growth, are the phosphate numbers of the same order.

Phosphate tests were made intermittently during a period of six months on milk samples from approximately 100 different herds. The purpose was to determine the variation, if any, in the reaction to the phosphate test in milk samples from the same herd collected at daily or weekly intervals. It was found that in a large majority of the herds the milk was fairly constant from day to day in its reaction to the phosphate test. The fact that the milk was phosphate positive on one day was fairly good assurance that it would be phosphate positive the day following. Milk from several herds was consistently phosphate positive for over a period of three months, while milk from other herds was phosphate negative during two or three months at a time. However, milk from a few herds (representing about 5 per cent of all studied) would fluctuate some from day to day in its reaction to the test, and others over longer intervals. These samples had phosphate numbers that varied between 20 and 22. While they were not consistent in their reaction to the test, their actual daily variation in phosphate resistance was small. Milk from one particular herd in which a study was made for over a year, was always of good quality in respect to its heat stability and the phosphate numbers were never less than 26. This herd consisted of registered Holstein cattle of very large frame. The cows were milked three times daily. Another herd gave milk throughout the year of low heat stability and of phosphate numbers consistently below 22. In general, milk from the same herd that reacts phosphate positive or phosphate negative on one day, is quite apt to react the same the day following and often one week later. However, it is suggested that tests should be made at least twice a month in order to maintain an adequate check.

Addition of disodium phosphate, to the milk that coagulated in the phosphate test always resulted in an improved product. On the other hand, milks of high phosphate numbers were generally reduced in their resistance to heat when treated with the disodium phosphate. Work is now in progress to determine what relationship does exist, if any, between phosphate numbers and amount of disodium phosphate to add for optimum stability.

DISCUSSION

A study of the results presented shows that milk coagulated in the phosphate test is of low heat stability. Furthermore, composites made up of samples of milk of this class are also of low heat stability. Likewise, elimination of the milks that respond to the phosphate test from the total supply, often results in obtaining a more stable product, and an increase in stability is invariably produced when the percentage of these milks represent

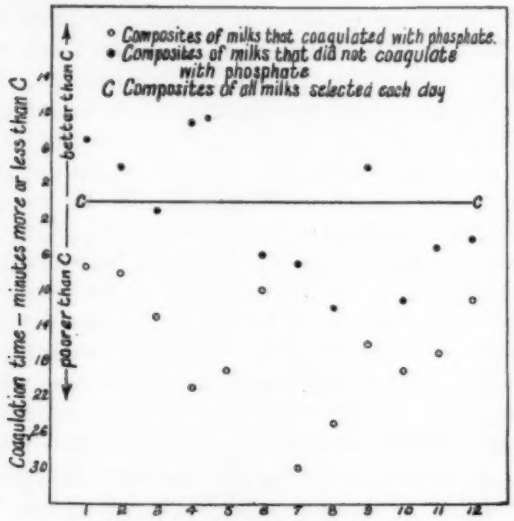

FIG. 3. COMPARATIVE STABILITY OF COMPOSITE SAMPLES OF CONDENSERY MILK SEPARATED ON BASIS OF PHOSPHATE TEST

over 50 per cent of the total milk under investigation. However, the heat stabilities of composites are not always increased by eliminating the samples that respond to the test, at least when the percentage of poor milks is small in comparison to the total amount. In fact, it was found that one batch was not improved at all by eliminating the poor stability milks. The poor milks comprised 45 per cent of the total amount studied on this date. Then again, another batch was increased 3 minutes in stability

TABLE 3

*Stability of composite samples of milk separated on basis of phosphate test, when using 10 cc. of milk and 1 cc. of phosphate solution in the test**

NUMBER	COMPOSITE	COMPOSITION OF COMPOSITES		COAGULATION TIME	CHANGE IN STABILITY FROM TOTAL COMPOSITE (C)
		A	B		
		per cent	*per cent*	*minutes*	*minutes*
1	A†	100	0	8	7−‡
	B	0	100	22	7+
	C	40	60	15	
2	A	100	0	2	8−
	B	0	100	14	4+
	C	33	66	10	
3	A	100	0	2	13−
	B	0	100	14	1−
	C	16	84	15	
4	A	100	0	1	21−
	B	0	100	31	9+
	C	8	92	22	
5	A	100	0	1	19−
	B	0	100	Lost	
	C	8	92	20	
6	A	100	0	5	10−
	B	0	100	9	6−
	C	37	63	15	
7	A	100	0	7	30−
	B	0	100	30	7−
	C	37	63	37	
8	A	100	0	8	25−
	B	0	100	21	12−
	C	33	67	33	
9	A	100	0	9	16−
	B	0	100	29	4+
	C	33	67	25	
10	A	100	0	11	19−
	B	0	100	19	11−
	C	33	67	30	

TABLE 3—*concluded*

NUMBER	COMPOSITE	COMPOSITION OF COMPOSITES		COAGULATION TIME	CHANGE IN STABILITY FROM TOTAL COMPOSITE (C)
		A	B		
		per cent	*per cent*	*minutes*	*minutes*
11	A	100	0	8	17—
	B	0	100	20	5—
	C	16	84	25	
12	A	100	0	18	11—
	B	0	100	25	4—
	C	25	75	29	

* Results obtained by B. H. Webb, in a condensery in California.
† A Composite of milks that coagulated with phosphate test.
B Composite of milks that did not coagulate with phosphate test.
C Composite of all milks selected each day.
‡ − Decrease in stability.
+ Increase in stability.

by separating out the poor milks which represented only 15 per cent of the total amount (by volume) used that day.

Webb[1] found somewhat similar results when applying the test at a condensery in California. His results are tabulated in table 3, and comparisons of his A and B composites with his C composites are shown in figure 3. The milk arriving at the condensery at that time was very stable and the rejection of the poor milks or the use of a neutralizer was seldom necessary. However, the A composites were always of lower heat stability than either the C or B composites, which is in accord with our findings. On several days Webb found that certain percentage mixtures of his A and B composites gave a more stable product than his B composites alone. In this connection, that is when mixtures of the A and B milks are made, a determination of the phosphate numbers of such mixtures will often aid in approximating their resultant stabilities. By this means, mixtures of the requisite heat stability may be obtained.

In view of the fact that elimination of poor stability milk does not always result in producing a resultant milk of higher stability,

[1] From an unpublished report made by Byron H. Webb, of this laboratory.

use of the test should be governed by the results obtained and consideration should be given to the type of milk arriving at the condensery. Its real purpose is to aid in picking out those milks that impair the stability of the total composite during the seasons when sterilization is giving trouble, and not to be used as a quality test of sanitary milk. The amount of solution to use in the test is likewise variable, and should be governed by the type of milk under examination. When the elimination of milk that coagulates when 1 cc. of the phosphate solution is treated with 10 cc. of milk does not produce an increased stability in the remaining batch, the use of lesser amounts might. The appropriate amount can be ascertained by trial only.

SUMMARY AND CONCLUSIONS

1. A test has been developed for the detection of milk unstable to heat. The test is rapid and made applicable to factory routine.

2. Milk that coagulates in the test is invariably of low heat resistance. Composites of this class of milk are also of low heat stability.

3. In grading milk on the basis of the phosphate test, the elimination of the phosphate positive milk often results in obtaining a milk of higher stability towards heat.

4. Phosphate number is defined.

5. Relation between phosphate number and coagulation time is shown.

6. There is no apparent relationship between pH and phosphate number.

7. Single herds are reasonably constant in their phosphate number.

8. This test has been tried on a commercial scale in a condensery in California.

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SPEED OF DASHER AND SCRAPER AS AFFECTING THE QUALITY OF ICE CREAM AND SHERBET*

E. L. REICHART

Department of Dairy Husbandry, University of Nebraska, Lincoln

INTRODUCTION

The texture of ice cream is largely determined by the structure of the ice crystals constituting the body or bulk of ice cream and the air spaces separating these crystals. The smoothness of ice depends upon controlling the crystal and air cell formation. Factors entering into the control of crystal and air cell structure may be classified into two general groups: First, ingredient factors (2, 5); and second, processing factors. It is with a phase of this second group of factors that these experiments deal.

It is well known that freezing conditions have a marked influence on the body and texture of the finished ice cream (1, 4). Of these, brine temperatures, sharpness of scraper blades, and the operation of the movable parts of the freezer are especially important (3). The major part of these trials deals with the operation of the scraper and dasher.

EQUIPMENT AND METHODS

The freezer used was a forty-quart Fort Atkinson, which had been rebuilt and equipped with several sets of different size gears. It was so arranged that the dasher could be operated independently from the scraper and a motor was attached to drive it independent of the scraper. A two-way switch was attached to the motor driving the dasher to provide for the operation of the dasher in either direction, i.e., opposite to the direction of the scraper as is normal, and in the same direction. By not turning on the dasher motor, it was also possible to have the dasher idling, being propelled in neither direction, but offering some resistance to the freezing mix due to its presence as an obstacle.

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The ice cream mix used in these trials contained 14 per cent fat, 10 per cent serum solids, 14 per cent sugar and 0.25 per cent gelatin, and was made from fresh cream, skim-milk, and condensed skim-milk (30 per cent serum solids). It was pasteurized at 145°F. for 30 minutes and homogenized with a two stage Colony homogenizer at 2000 and 1000 pounds pressure, cooled immediately to between 40 and 50°F. and then frozen with various times of aging.

TABLE 1

Influence of speed of dasher upon freezing ice cream

Date of experiment: October 2, 1929. Mix composition: Fat, 14 per cent; serum solids, 10 per cent; sugar, 14 per cent; gelatin, 0.25 per cent. Hardening room temperature: −5°F.

SAMPLE	FREEZING CONDITIONS	GEAR RATIO DASHER	DASHER SPEED	OVERRUN AT MINUTES INDICATED						BRINE TEMPERATURE	AGE OF MIX
				3	5	7	9	11	13		
			r.p.m.							°F.	hours
1	Scraper normal (295 r.p.m.) dasher high speed	94–21	260	45	55*	72	85	100		−15	40
2	Scraper normal (295 r.p.m.) dasher high speed	94–21	260	60	70*	70	75	85	100	−15	40
3	Scraper normal (295 r.p.m.) dasher low speed	94–14	163	80	70*	72	81	90	95	−13	42
4	Scraper normal (295 r.p.m.) dasher low speed	94–14	163	70	65*	70	81	94	100	−13	42
5	Scraper normal (295 r.p.m.) dasher medium speed	94–16	200	60	70*	70	79	95	110	−15	72
6	Scraper normal (295 r.p.m.) dasher medium speed	94–16	200	50	59*	68	80	96	100	−15	72

* Time when brine was shut off.

Experimental work

Trial I. Variations of dasher speed. This trial consisted of three duplicate runs with the dasher operated at high, low, and medium speed, respectively. At high speed the dasher was turning 260 revolutions per minute; at medium speed (normal), 200; and at low speed, 163 revolutions per minute. The scraper was operated at normal speed (295 r.p.m.). Overrun readings

were taken as indicated with the attempt made to freeze all mixed to 100 per cent overrun.

Samples from the above batches were placed in the hardening room, allowed to harden for 48 hours, and then examined for body and texture differences by four members of the department. It was their unanimous opinion that there were no appreciable differences in the texture or body of the six different samples as shown in table 1. Figures in the overrun column indicate that there was very little difference in the length of time necessary either to freeze the ice cream sufficiently or to incorporate 100 per cent overrun. These trials seemed to indicate that the variation of the dasher speed from 163 to as high as 260 revolutions per minute had no marked effect on the finished product, the freezing time or the incorporation of overrun in a mix of the above composition.

Trial II. It was thought however, that in freezing sherbets, especially where difficulty frequently was encountered in keeping down the overrun, that the speed of the dasher might be the cause of the difficulty. With this idea in mind, a series of trials with sherbets were planned and carried out as shown in table 2.

In table 2 are presented the results of 20 trials with sherbet. It is a well-known fact that it is easy to obtain a large overrun in freezing sherbet, while only a moderate overrun is desired. Experience had indicated that under ordinary circumstances an overrun of between 30 and 40 per cent gives most satisfactory results, because of the better body. The first 8 samples represent trials with the scraper operating at normal speed, while the dasher was operated at high speed. The desired overrun was obtained at between 7 and 9 minutes and after that the whipping incorporated too much air. The second set of trial samples, 9 to 16 inclusive, had the scraper operating at normal and the dasher operating at normal or medium. In these trials the desired overrun was obtained in the same time, but there was not the excessive whipping afterward. In the third set of trials, samples 17 to 24 inclusive, the scraper was operated at normal speed, and the dasher was operated at slow speed. Here the overrun was incorporated somewhat slower throughout the entire whip-

TABLE 2

Influence of dasher speed upon freezing sherbet

Date of experiment: October 8, 16, 30, November 4, 1929. Mix composition: Sugar, 27 per cent; serum solids, 0.032 per cent; stabilizer, 0.0068 per cent. Hardening room temperature: −5°F.

SAMPLE	FREEZING CONDITIONS	GEAR RATIO DASHER	DASHER SPEED	OVERRUN AT MINUTES INDICATED						BRINE TEMPERATURE
				5	7	9	11	13	15	
			r.p.m.							°*F.*
1	Scraper normal (295 r.p.m.) dasher high speed	94–21	260	15	25	35	70	110	140	−5
2	Scraper normal (295 r.p.m.) dasher high speed	94–21	260	15	25	35	75	115	120	−5
3	Scraper normal (295 r.p.m.) dasher high speed	94–21	260	14	28	36	70	110	130	−5
4	Scraper normal (295 r.p.m.) dasher high speed	94–21	260	16	24	38	80	120	135	−5
5	Scraper normal (295 r.p.m.) dasher high speed	94–21	260	12	22	30	65	100	120	−5
6	Scraper normal (295 r.p.m.) dasher high speed	94–21	260	15	25	35	70	105	140	−5
7	Scraper normal (295 r.p.m.) dasher high speed	94–21	260	18	28	40	75	115	160	−5
8	Scraper normal (295 r.p.m.) dasher high speed	94–21	260	15	25	35	70	110	120	−5
Average				15	25.2	35.5	71.8	110.6	133.1	−5
9	Scraper normal (295 r.p.m.) dasher medium	94–16	200	15	20	30	60	75	89	−5
10	Scraper normal (295 r.p.m.) dasher medium	94–16	200	15	23	35	58	80	95	−5
11	Scraper normal (295 r.p.m.) dasher medium	94–16	200	16	20	35	60	70	86	−4
12	Scraper normal (295 r.p.m.) dasher medium	94–16	200	15	25	40	50	80	94	−4
13	Scraper normal (295 r.p.m.) dasher medium	94–16	200	14	20	38	45	75	75	−4
14	Scraper normal (295 r.p.m.) dasher medium	94–16	200	16	24	36	52	85	88	−3
15	Scraper normal (295 r.p.m.) dasher medium	94–16	200	15	22	35	54	80	82	−3
16	Scraper normal (295 r.p.m.) dasher medium	94–16	200	18	28	40	50	75	80	−3
Average				15.5	22.7	36.1	53.6	77.5	86.1	−3.8

17	Scraper normal (295 r.p.m.) dasher slow	94–14	163	10	15	20	25	27	45	−5
18	Scraper normal (295 r.p.m.) dasher slow	94–14	163	10	15	28	30	35	60	−5
19	Scraper normal (295 r.p.m.) dasher slow	94–14	163	10	14	34	35	40	56	−5
20	Scraper normal (295 r.p.m.) dasher slow	94–14	163	8	15	27	32	42	50	−5
21	Scraper normal (295 r.p.m.) dasher slow speed	94–14	163	9	16	30	40	50	65	−5
22	Scraper normal (295 r.p.m.) dasher slow speed	94–14	163	10	14	25	28	38	48	−5
23	Scraper normal (295 r.p.m.) dasher slow speed	94–14	163	10	15	28	34	40	60	−4
24	Scraper normal (295 r.p.m.) dasher slow speed	94–14	163	10	14	24	38	44	65	−3
Average				9.6	14.7	27	32.7	39.5	56.1	−4.6
25	Scraper normal (295 r.p.m.) dasher idle			10	12	20	24	35	40	−4
26	Scraper normal (295 r.p.m.) dasher idle			10	14	25	27	32	30	−4
27	Scraper normal (295 r.p.m.) dasher idle			10	14	22	24	34	35	−4
28	Scraper normal (295 r.p.m.) dasher idle			10	14	23	24	30	32	−3
29	Scraper normal (295 r.p.m.) dasher idle			10	12	21	26	35	32	−4
30	Scraper normal (295 r.p.m.) dasher idle			8	14	20	25	30	35	−3
31	Scraper normal (295 r.p.m.) dasher idle			8	12	24	28	30	35	−3
32	Scraper normal (295 r.p.m.) dasher idle			8	10	20	24	32	30	−3
Average				9.2	12.7	21.8	25.2	32.2	33.6	−3.5

ping period and the maximum obtained was much lower. The last series of trials, samples 25 to 32 inclusive—were run with the scraper normal and the dasher idle. In these trials the overrun was obtained slower than in the other trials and the maximum reached was less. As the mix is usually frozen sufficiently to draw in 9 minutes, it is apparent that operating the dasher at a low speed or idle, assists in keeping the overrun down to a desirable amount for sherbet. Where brine temperatures are not low enough to complete freezing by the time the desired overrun has been reached, the time necessary to complete freezing usually allows too much whipping unless the dasher is operated slower than normal. These trials raise the question of the desirability of having a variable speed dasher in brine freezers used for making sherbets.

Trial III. A study of table 1 indicates that the variations in the dasher speed had but little effect upon the rapidity of obtaining overrun or upon the body and texture of the finished ice cream. This suggested that the scraper speed might be of greater influence than the dasher speed in obtaining more rapid incorporation of overrun.

In order to determine the effect of the scraper as compared with the dasher upon the freezing of ice cream, a series of runs was made in which the dasher was operated at normal speed, while the scraper was operated at different speeds. The same composition mix was used as in the previous trials with ice cream. In table 3 the results obtained are presented in tabular form.

In table 3 samples 1 and 2; and 6, 7, and 8 were frozen with scraper and dasher operating at normal speeds. The desired overrun, 100 per cent was obtained in 13 minutes in each case. Samples 3, 4, and 5; and 9, 10, and 11 were frozen with dasher operating at normal speed, but with the scraper speeded up 35 revolutions per minute. In these runs the desired overrun was obtained in 9 minutes with 4 samples and in 11 minutes with 2 samples. There was a saving of from 2 to 4 minutes in freezing time. These samples were hardened and then scored by four members of the department and a slightly smoother body was observed in the samples frozen at a high scraper speed. The

TABLE 3

Influence of scraper speed upon freezing ice cream

Date of experiment: October 8, 1929. Mix composition: Fat, 14 per cent; serum solids, 10 per cent; sugar, 14 per cent; gelatin, 0.25 per cent. Hardening room temperature: −3°F.

SAMPLE	FREEZING CONDITIONS	GEAR RATIO SCRAPER	SCRAPER SPEED	OVERRUN AT MINUTES INDICATED						BRINE TEMPERATURE	AGE OF MIX
				3	5	7	9	11	13		
			r.p.m.							°F.	hours
1	Scraper normal, dasher normal (200 r.p.m.)	83–21	295	60	70	70*	79	95	110	−15	72
2	Scraper normal, dasher normal (200 r.p.m.)	83–21	295	50	59*	68	80	96	100	−15	72
3	Scraper fast, dasher normal (200 r.p.m.)	53–15	330		58*	71	90	105		−12	72
4	Scraper fast, dasher normal (200 r.p.m.)	53–15	330	50	67*	96	101			−12	72
5	Scraper fast, dasher normal (200 r.p.m.)	53–15	330		43*	90	100			−12	72
6	Scraper normal, dasher normal (200 r.p.m.)	83–21	295	61	70*	70	75	88	100	−10	72
7	Scraper normal, dasher normal (200 r.p.m.)	83–21	295	55	65*	72	78	90	101	−10	72
8	Scraper normal, dasher normal (200 r.p.m.)	83–21	295	50	58*	68	80	95	100	−10	72
9	Scraper fast, dasher normal (200 r.p.m.)	53–15	330	46	60*	75	94	103		−10	72
10	Scraper fast, dasher normal (200 r.p.m.)	53–15	330	52	68*	90	100			−10	72
11	Scraper fast, dasher normal (200 r.p.m.)	53–15	330	51	72*	85	98			−10	72

* Time when brine was shut off.

saving of time appears to be advantageous from a commercial standpoint, especially when there apparently is a slight improvement in body and texture. The operation of the scraper at a speed higher than normal tends however to create so much centrifugal force within the freezer that it is difficult to get the mix into the freezer from the hopper without discontinuing the operation of the scraper.

TABLE 4

Influence of scraper and dasher upon freezing ice cream

Date of experiment: October 16, 1929. Mix composition: Fat, 14 per cent; serum solids, 10 per cent; sugar, 14 per cent; gelatin, 0.25 per cent. Hardening room temperature: −5°F.

SAMPLE	FREEZING CONDITIONS	GEAR RATIO DASHER	DASHER SPEED	OVERRUN AT MINUTES INDICATED					BRINE TEMPERATURE	AGE OF MIX
				3	5	7	9	11	13	
			r.p.m.						°F.	hours
1	Scraper fast (330 r.p.m.) dasher idle				48*	70	87	94	99	−10	24
2	Scraper fast (330 r.p.m.) dasher idle				45*	60	84	93	98	−10	24
3	Scraper fast (330 r.p.m.) dasher idle				46*	72	83	95	98	−8	24
4	Scraper fast (330 r.p.m.) dasher idle				48*	74	86	95	100	−8	24
5	Scraper fast (330 r.p.m.) dasher same direction as scraper	94–16	200		51*	65	79	88	96	−8	28
6	Scraper fast (330 r.p.m.) dasher same direction as scraper	94–16	200		51*	65	82	95	99	−8	28
7	Scraper fast (330 r.p.m.) dasher same direction as scraper	94–16	200		50*	65	80	96	100	−8	28

* Time when brine was shut off.

Trial IV. In order to further check the influence of the dasher upon the freezing of ice cream, runs were made with the dasher either idling, or operating in the same direction as the scraper, at medium or normal speed, while the scraper was operated at high speed. The results are presented in table 4.

The time required to obtain satisfactory overrun was uniformly 13 minutes in these runs. This is the same time that was needed when the dasher speed was high, and the scraper normal, (table 1, sample 2, (sample 1 froze in 11 minutes); dasher speed low, scraper

normal, table 1, samples 3 and 4; dasher speed normal and scraper normal, table 1, samples 5 and 6). This seems to indicate that within the speed limits used, the speed of the dasher has no marked influence upon the time necessary to incorporate desired overrun. The mix used in this trial was identical in composition with that used in previous trials and the resulting ice cream after hardening was pronounced no different in texture and body, by four members of the department, from the ice cream produced in the other trials.

SUMMARY

1. The speed of the dasher (200 r.p.m. normal; 163 r.p.m. low; 260 r.p.m. high) has no appreciable effect upon the rapidity of freezing ice cream; upon the rapidity of incorporating 100 per cent overrun; or upon the body and texture of the finished ice cream, when made from fresh dairy products.

2. In freezing sherbets, where an overrun in excess of 30 to 40 per cent is undesirable, the reduction of the effect of the dasher by reducing its speed, by allowing it to idle, or by operating at normal speed in the same direction as the scraper, is useful in preventing the too rapid incorporation of overrun and the consequent necessity of drawing the sherbet before it is properly frozen.

3. The operation of the scraper at a speed above normal slightly shortens the freezing time and also the time necessary to incorporate 100 per cent overrun and tends to produce a slightly smoother body.

4. Comparing the effect of variations of speed of dasher and scraper in freezing ice cream, the scraper is the more important.

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AN INTERPRETATION OF THE FEEDING STANDARDS FOR GROWING DAIRY CATTLE*

J. B. FITCH

Department of Dairy Husbandry, Kansas Agricultural College, Manhattan

AND

R. H. LUSH

Agricultural Experiment Station, Louisiana State University, Baton Rouge

In experimental work in feeding growing dairy animals over long periods of time, there is a need for definite standards of feed requirements. It is necessary to use some guide; first, to insure that animals receive sufficient amounts of nutrients and, second, in order that trials may be accurately repeated. It is realized that any arbitrary standard is likely to be but an approximation of requirements for growth, especially if little or no attempt is made to consider the source of energy, the quality of the protein, or the mineral and vitamin content of the food. The authors do not propose to criticize existing standards but, rather, to present some experience in interpreting them, believing that other workers may have had the same difficulty. Table 1 shows the requirements as listed in two standard texts.

The Armsby (1) standard expresses in digestible true protein and net energy the requirements for animals of different weights. No figures are listed for animals weighing over 800 pounds. The usefulness of the Armsby standard is limited by the fact that the net energy is not known for all feeds.

The Morrison (2) standard is adaptable to more general use, especially in experimental trials with new feeds or those of varying composition that can readily be analyzed. Also it can be used for larger animals than can the Armsby standard. The Morrison standard is used almost exclusively at this Station for these reasons. However, in figuring the nutrients required for an ani-

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mal of a given weight, inconsistencies are encountered because of the abrupt change in requirements from one 100 pound class to the next. The requirements for both protein and total digestible nutrients are expressed in maximum and minimum pounds required for 100 pounds live weight. The range in protein is given in order that the feeder may take advantage of fluctuations in price of protein feeds. However, to get the average requirement for experimental work, one would ordinarily average the two extremes of the class, divide by 1000 and multiply by the actual weight of the animal. This is accurate for an animal weighing

TABLE 1

Nutrient requirements for growing dairy animals

WEIGHT	MORRISON, PER DAY PER 1000 POUNDS		ARMSBY, PER DAY		
	Digestible crude protein	Total digestible nutrients	Weight	Digestible true protein	Net energy
pounds	*pounds*	*pounds*	*pounds*	*pounds*	*therms*
100–200	2.9–3.2	17.0–19.0	100	0.40	3.1
200–300	2.6–2.9	16.5–18.5	135	0.45	3.4
300–400	2.3–2.6	15.5–17.5	165	0.55	3.6
400–500	2.0–2.3	14.5–16.5	275	0.70	4.1
500–600	1.8–2.0	13.8–15.8	325	0.75	4.4
600–700	1.7–1.9	13.0–15.0	400	0.80	5.1
700–800	1.6–1.8	12.2–14.2	550	0.85	6.4
800–900	1.5–1.7	11.4–13.4	700	0.85	7.6
900–1,000	1.3–1.5	10.6–12.6	800	0.85	8.2

near the middle of the weight class. But using the same method, it will be found that an animal weighing near the upper limit of one class will be shown to require more protein than another animal weighing near the lower limit of the next higher class. That is to say the feed requirement so calculated will be least for the heavier animal. This variation is illustrated by the dotted line in figure 1, in which the maximum, minimum, average, and calculated amounts of protein required for animals weighing from 150 to 950 pounds is plotted. The average which is based on the maximum and minimum for each 50 pound class accurately represents the protein requirements for any size animal. Where

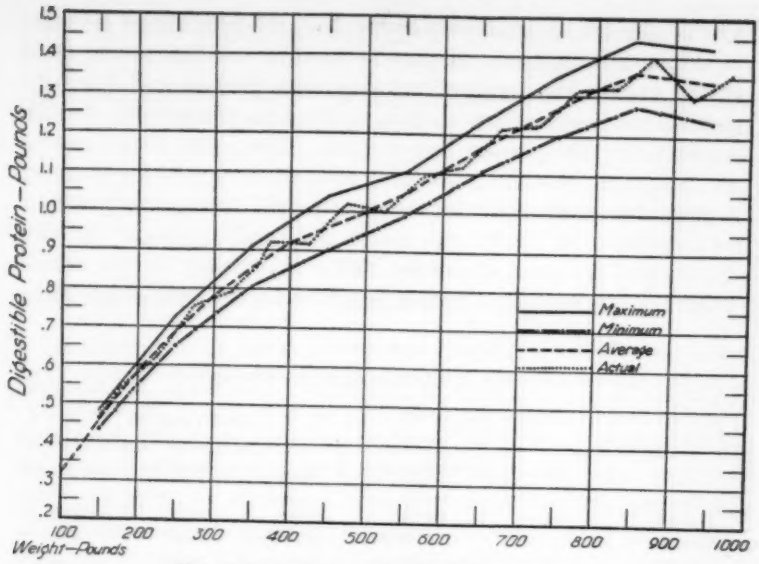

FIG. 1. DIGESTIBLE PROTEIN REQUIREMENTS

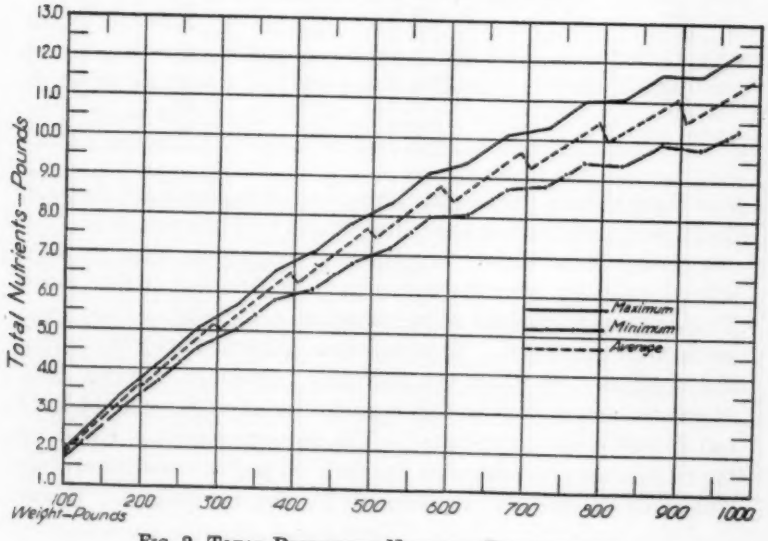

FIG. 2. TOTAL DIGESTIBLE NUTRIENT REQUIREMENTS

this curve crosses a base line of a given weight, the digestible protein required will be found expressed in the legend on the left. This curve is satisfactory until 900 pounds is reached. For animals reaching maturity at about 950 pounds as with the Jersey breed this figure may be correct as the protein requirement de-

TABLE 2

Morrison Standard for Growth (with suggested changes)

WEIGHT	DIGESTIBLE CRUDE PROTEIN	TOTAL DIGESTIBLE NUTRIENTS
pounds	*pounds*	*pounds*
100	0.320	1.9
150	0.457	2.70
200	0.580	3.55
250	0.687	4.375
300	0.780	5.10
350	0.857	5.775
400	0.920	6.40
450	0.967	6.975
500	1.00	7.575
550	1.045	8.14
600	1.11	8.64
650	1.17	9.10
700	1.225	9.52
750	1.275	9.90
800	1.320	10.24
850	1.335	10.54
900	1.350	10.80
950	1.365	11.02
1,000	1.37	11.20
1,050	1.38	11.34
1,100	1.39	11.44
1,150	1.41	11.50
1,200	1.42	11.53

pends on rate of growth and maintenance. But from Armsby (3) and from Morrison's standard for growing fattening steers it is reasonable to believe that the total amount of protein used increases slightly with any considerable increase in body weight. On this assumption we have completed the curve of requirements to 1200 pounds using the rate of regression from 100 to 800 pounds. An animal of each 50 pound class requires a smaller percentage of protein than the preceding class until the increase amounts

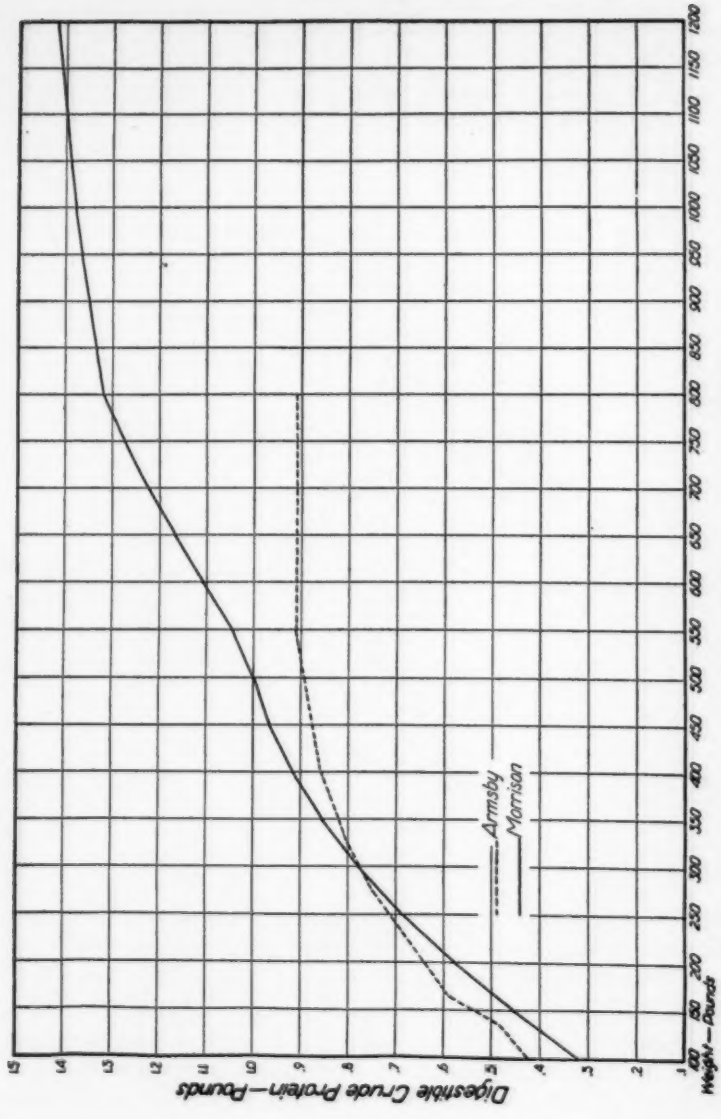

FIG. 3. COMPARISON OF ARMSBY AND MORRISON PROTEIN REQUIREMENTS FOR GROWTH

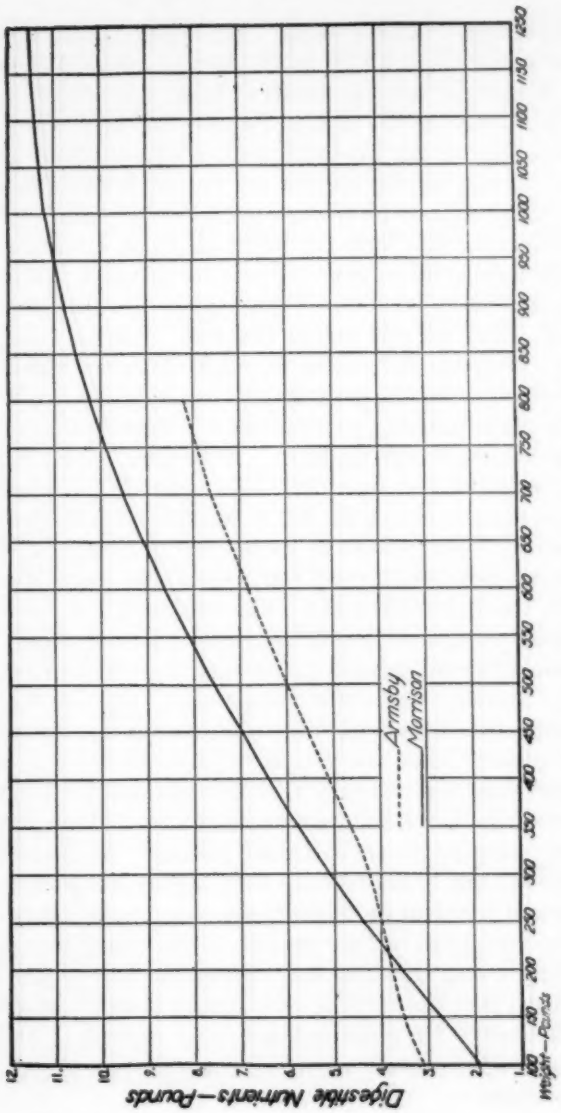

FIG. 4. COMPARISON OF ARMSBY AND MORRISON TOTAL NUTRIENT REQUIREMENT FOR GROWTH

to only 0.7 per cent at 1200 pounds. These percentage increases are slightly smaller than for growing fattening steers of the same weight. This curve, then, as shown in figure 3 is probably conservative in estimating the protein required for large dairy animals. According to the Missouri Station (4), Holstein heifers required an average of only 69.9 per cent of the Wolff-Lehnmann (Morrison) requirements for protein while Jerseys required 101.6 per cent. Granted that there is a breed difference in metabolism due to a difference in the ratio of body surface to weight and other possible factors, the 1000 pound Holstein heifer should have more total protein than the 900 pound Holstein. Similar reasoning should hold for other differences in weight between animals of the same body type or else maintenance requirements are wrong.

Much the same difficulty is encountered in figuring the amount of total digestible nutrients required. If the table is used literally an animal weighing just over 600 or 700 pounds, for example, would require less nutrients than one weighing slightly less than these amounts. This is shown by the dotted line in figure 2, which shows the total digestible nutrient requirements for animals weighing between 100 and 975 pounds. The maximum and minimum requirements were figured for animals of each 25 pound class. Certainly an animal's requirements do not vary as markedly as shown here nor did Morrison make that assumption. But the only fair way to interpret the figures for ready use is by taking averages for 50 and 100 pound classes and plotting a curve as shown in figure 4. The nutrients required may then be read directly from the intersection of the curve and base or weight line. Here again it is necessary to extend the curve to 1200 pounds. As the increase in nutrients of each 50 pound class from 200 to 950 pounds was 0.04 of a pound less than the increase over the previous class, this rate was used from 950 to 1200 pounds. The rate of increase in nutrients for growing fattening steers is considerably higher than this, indicating that the curve is very conservative in estimating nutrients necessary for growing large dairy animals. Table 2 presents the original data from which the solid line of figures 3 and 4 are plotted.

We do not have sufficient data to say whether the Morrison

standard would supply enough nutrients for normal or average growth. Normal growth itself is affected by many local factors. This standard is generally believed to be liberal in its allowance of nutrients, however, and work of the Missouri Station indicates this to be true. In figures 3 and 4 is presented a comparison of the Armsby and Morrison standards for growth, in terms of the latter. Corn was selected as a basis for converting digestible true protein to terms of digestible crude protein. For example, in the case of a 410 pound animal, 0.8 pounds of digestible true protein is required according to Armsby. Dividing 0.8 by 0.07, the amount of digestible true protein in one pound of corn, the result indicates that 11.42 pounds of corn would be necessary to supply this protein. Then by multiplying by 0.075 the equivalent amount of digestible crude protein is found to be 0.857 pounds. This figure, then, represents the Armsby requirement expressed in the Morrison term. Similarly, all the stated requirements were converted and listed in figure 3.

The curve given then represents the Armsby figures for protein on a concentrate basis with no attempt to correct or extend it. The same process was used in converting net energy to equivalent figures of total digestible nutrients in figure 4. Here the difference is not so marked. But with alfalfa hay as a basis for comparison, the Armsby standard is close to or exceeds that of Morrison. That is, an 800 pound dairy heifer fed on alfalfa hay alone according to net energy requirements will get more total digestible nutrients and protein than called for in the Morrison standard. The choice of a standard or guide to follow will depend somewhat on the type of experimental feed used.

SUMMARY

Any feeding standard should be looked upon only as a guide. The Morrison standard for growing dairy animals, however, can be readily used in the majority of experimental trials and it is hoped more quickly interpreted by the use of table 2 plotted on a large scale as shown in figures 3 and 4.

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THE EFFECT OF FEEDING MENHADEN (FISH) OIL ON THE SECRETION OF MILK AND THE COMPOSITION OF BUTTER FAT IN THE DAIRY COW*

J. B. BROWN AND T. S. SUTTON

Laboratory of Physiological Chemistry and the Department of Animal Husbandry, Ohio State University, Columbus

The problem of the effect of diet on the quality and quantity of milk from the dairy cow has been one of great interest and the subject of numerous investigations, including those of Wing (1), Anderson (2), Eckles and Palmer (3), Mattick (4), Petersen (5), Maynard and MaCay (6) and Petersen, Palmer and Eckles (7). Milk as a glandular secretion and as a food is unique not only because it contains at least two biologically complete proteins, casein and lactalbumin, and a characteristic sugar, lactose, but also because it contains a fat that is composed of glycerides of oleic acid and of the entire series of saturated fatty acids up to stearic. Few of these compounds are ordinarily found in the food of the cow. Casein, lactalbumin, lactose and the lower fatty acids are apparently, therefore, products of the synthetic action of the mammary gland. The higher fatty acids, palmitic, stearic and oleic, appear to be derived for the most part from the blood lipids and hence from the fat of the diet.

In a continuance of a series of investigations on the fate of highly unsaturated fatty acids in animal metabolism (8), the primary object of the present investigation was to trace definitely the passage of the characteristic highly unsaturated fatty acids of menhaden (fish) oil from the food into butter fat in the dairy cow. These acids consist of a mixture of the following (9):

Hexadecatrienoic	$C_{16}H_{26}O_2$
Clupanodonic	$C_{18}H_{28}O_2$
Arachidonic	$C_{20}H_{32}O_2$
Eicosapentenoic	$C_{20}H_{30}O_2$
Docosapentenoic	$C_{22}H_{34}O_2$
Docosahexenoic	$C_{22}H_{32}O_2$

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They may be easily identified by bromination as acids or methyl esters in cold ether, when white, insoluble polybromides are formed which may be analysed for bromine, and which, in the case of the methyl esters of the lower members of the series up to arachidonic, give characteristic melting points. These acids, further, are non-toxic and are deposited readily in the body fat of the rat (8) and the hog (10) when they are included in the diet. One of them, arachidonic, occurs normally in rat fat (11), human fat (12), and lard (13) in very small quantities. Comparatively large amounts of it occur in the lipids of the brain and some of the glandular tissues.

A number of investigators have demonstrated the passage of abnormal fatty acids into butter fat when these acids are included in the diet. Caspari (14) found iodized fat in butter when the iodized fat was fed. Engel (15) claims to have found sesame oil in human milk after the oral ingestion of this oil. Bowes detected arachidic acid ($C_{20}H_{40}O_2$) in goat butter twelve hours after peanut oil was eaten (16). Gogitidse (17) found that sheep after eating linseed oil gave milk fat containing as much as 33 per cent of the oil, and that this oil readily passed into human milk. Hempseed fat greatly depressed lactation. Channon, Drummond and Golding (18) have recently studied the effect of cod liver oil on lactation and quality of butter. In general their findings are confirmed by the work reported below. It should be noted that chemically the cod liver oil used by these investigators is very similar to menhaden oil.

In the present investigation a dairy cow after a six-day control period was fed on a diet containing considerable quantities of menhaden oil for fourteen days. After this period of experimental feeding the control diet was resumed for ten days. The general results of the fish oil feeding were to greatly reduce the milk production, the percentage of butter fat in the milk and total butter fat yield. Upon discontinuing the oil, partial recovery in milk yield occurred with complete recovery in the percentage of butter fat. As a result of chemical analysis of the butter fat samples, it was found that the normal butter of this cow yielded small amounts of ether insoluble bromine addition products, a result

which was confirmed by the analysis of several specimens of commercial butter. A small amount of a highly unsaturated fatty acid occurs, therefore, in normal butter. This acid may be arachidonic, or a mixture of arachidonic with other acids and has not been described previously. During the period of fish oil feeding the quantity of polybromides obtained increased about six times, showing that small amounts of the fish oil acids passed into the butter.

EXPERIMENTAL PART

Details of feeding

The feeding experiment was not completely satisfactory, mainly because of difficulty in getting the cow to eat the menhaden oil diet. Since, however, the main object of the work as stated above was attained, the results will be reported.

A purebred Holstein, weighing 1340 pounds in her sixth month of lactation, was used in the experiment. For the preceding month she had averaged 18.5 pounds of milk per day, testing 3 per cent butter fat. For the first six days of the experiment she was fed 6 pounds of basal mixture (Basal ration: 9 parts dried distillers grain, 5 hominy feed, 2 oats, 2 bran), $3\frac{3}{5}$ pounds of starch per day, and in addition 6 pounds beet pulp, $\frac{1}{2}$ pound molasses and no. 1 green alfalfa hay ad libitum. Milk samples were collected for two day periods, tested for butter fat, and separated. After six days of control ration (3 samples) $1\frac{1}{2}$ pounds of the menhaden oil (a commercial specimen of dark amber color of not unpleasant taste, except for its decided fishy odor) was substituted for the starch, the rations being calorifically isodynamic. Difficulty was experienced in getting the cow to take the feed. The oil in the ration was then lowered until the cow found the mixture palatable, with an attempt to keep the ration of equal calorific value by increasing the allowance of molasses and beet pulp which also aided in making the food succulent. The animal was kept on the fish oil diet for fourteen days. Toward the end of the feeding period she became such a slow eater that corn silage was used in replacing part of the beet pulp, thus slightly increas-

ing the protein content of the ration. Since the oil consumption was necessarily variable the average daily consumption is shown in figure 1. It should be noted in this chart, as in other charts and tables given below, that the sample numbers represent two-day specimens. After fourteen days of oil feeding, the oil was withdrawn from the ration and starch again added. The effect was followed for an additional ten days.

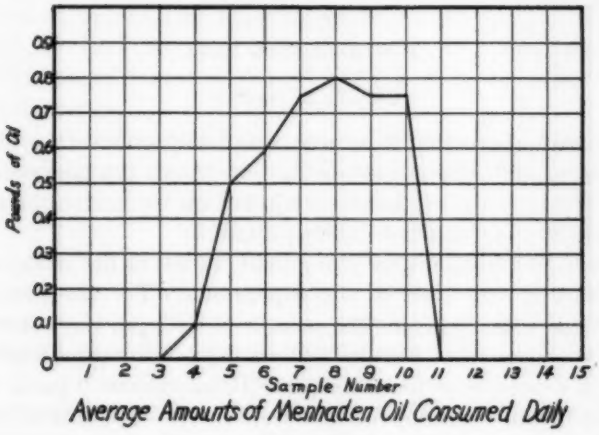

FIG. 1

Methods of Analysis

Butter fat was estimated by the Babcock method. The cream from two-day specimens of milk was worked up for the butter fat, which was dried by warming under reduced pressure and filtered through cotton to remove any particles of curd. Iodine, saponification, and Reichert-Meissl numbers were determined on the fat by the usual methods. The remainder of the butter fat after analysis was converted into methyl esters by boiling eighteen hours with an excess of dry methyl alcohol containing HCl gas in solution. The esters were distilled under reduced pressure (10 mm.) and analysed. The remainder of the esters was brominated in cold ether, the ether-insoluble bromides removed by centrifugation, and thoroughly washed in the centrifuge bottle with

fresh cold ether. They were weighed and the polybromide number (per cent yield of polybromides) was calculated. Bromine determinations were made by the Parr peroxide bomb method. Such small amounts of bromides were obtained from the control samples 1, 2, and 3 that these were combined for bromine analysis.

DISCUSSION OF RESULTS

Effect of milk production (fig. 2)

The daily milk production during the six-day control period was at the 17–18-pound level. This decreased rapidly to 10.4

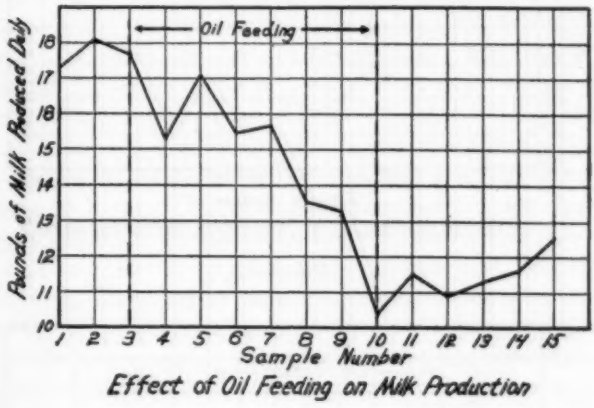

FIG. 2

pounds on the fish oil diet. Upon discontinuing the oil, recovery was slow, the output being only 12.5 pounds ten days after. Two weeks after the end of the experiment on the regular herd ration the yield was still only 12.1 pounds. A permanent drying off effect resulted under this experimental regime.

Effect on percentage of butter fat and total butter fat (figs. 3 and 4)

During the fish oil feeding period the butter fat dropped from 3.0 to 2.1 per cent, with almost complete recovery during resumption of the control ration. Two weeks later butter fat was normal.

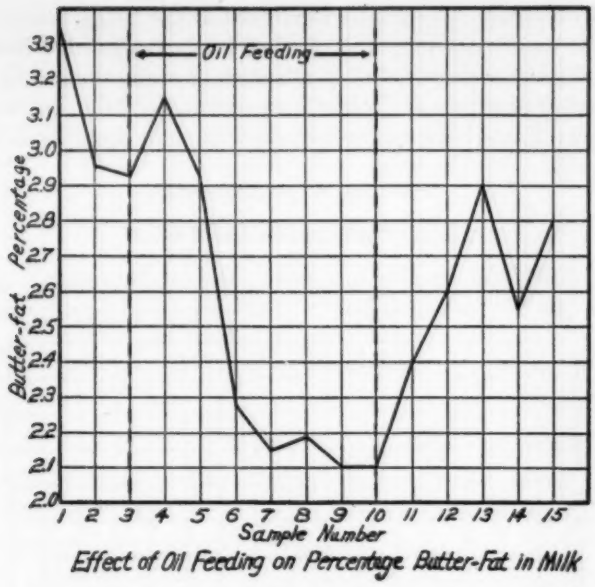
Oil Feeding
Butter-fat Percentage
3.3
3.2
3.1
3.0
2.9
2.8
2.7
2.6
2.5
2.4
2.3
2.2
2.1
2.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Sample Number
Effect of Oil Feeding on Percentage Butter-Fat in Milk

FIG. 3

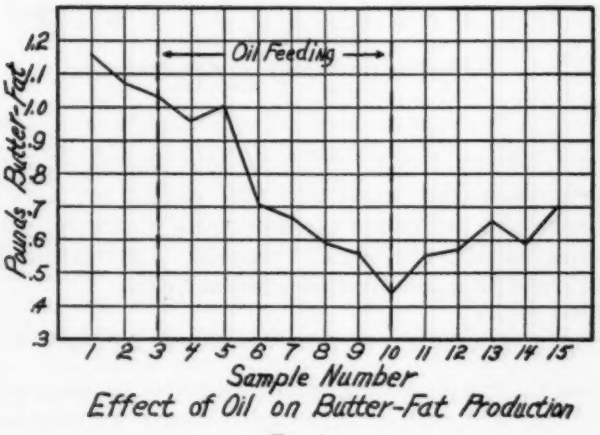
Oil Feeding
Pounds Butter-Fat
1.2
1.1
1.0
.9
.8
.7
.6
.5
.4
.3
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Sample Number
Effect of Oil on Butter-Fat Production

FIG. 4

The effect of the oil on total butter fat production was even more marked with a drop from 1.1 pound of fat per day to 0.45 pound. The butter fat yield did not recover completely after fish oil was discontinued because of the drying-off effect mentioned previously.

The data available are not sufficient to explain this very remarkable effect. Three causes might be given for the effect of the oil on the quality and quantity of the milk. (*a*) A relatively high fat content of the diet. This question has been discussed many times by previous investigators with no final conclusions. (*b*) The high vitamin content of the oil, especially vitamin D. While menhaden oil contains only about one-half the vitamin D of cod liver oil, the amount of this vitamin ingested was quite considerable and might account for the change. This specimen of oil was not tested for vitamin D. (*c*) The effect of the highly unsaturated acids. However, no deleterious physiological action has been observed previously on feeding menhaden oil. Rats have been fed on diets containing up to 30 per cent of this oil with no apparent harm. Our data offer no means of deciding these questions although we feel that D hyper-vitaminosis is the most likely cause. They are important not only to the dairyman but also to those interested in the nutrition of nursing women. The prescribing of cod liver oil and of viosterol (irradiated ergosterol, vitamin D) is a common practice with the medical profession. The effect of large doses of viosterol on the secretion of human milk may be very important and even harmful.

Chemical changes in the butter fat (table 1)

Chemically the butter fat during the control period was not unusual, except for the slightly higher than average iodine number. The results in table 1 are in agreement with the hypothesis that the fatty acid radicals of the food pass into the butter, appearing therein as glycerides. The body processes which account for the synthesis of the lower saturated fatty acids are probably not greatly modified. Instead, more molecules of higher fatty acids are present in the blood and in increased amounts pass into the butter. The result, therefore, of feeding an oil with a high content of unsaturated fish oil fatty acids with high iodine number

and molecular weight is to raise the iodine number of the butter as much as 20 points (50 per cent increase), to lower the saponification number, hence raise the molecular weight of the acids and to greatly lower the content of volatile soluble acids by increasing the content of the higher acids. The analytical data on sample (7), for example, were so changed from the normal that analyti-

TABLE 1

Analyses of butter

SAMPLE	IODINE NUMBER	SAPONIFICATION NUMBER	REICHERT-M NUMBER
1	41.40	224.68	25.9
2	41.89	224.22	25.0
3	42.42	224.69	26.7
Fish oil begins			
4	45.89	222.35	23.1
5	48.52	222.63	23.4
6	62.78	218.02	20.5
7	59.71	219.18	17.7
8	62.41	218.35	17.6
9	60.45	219.65	19.6
10	57.65	217.36	19.0
Fish oil ends			
11	53.49	214.60	17.0
12	47.78	215.68	17.1
13	43.76	217.30	17.9
14	41.87	217.93	19.0
15	40.67	220.92	20.3
Menhaden oil	176.61	187.5	1.64

cally the fat was not butter fat. This specimen actually remained liquid in the laboratory for several days. A very marked but not complete return of analytical data to normal occurred after the oil feeding was stopped.

The methyl ester analyses (table 2) are significant mainly with respect to polybromide number and percentage of bromine in the bromide determinations. We were surprised to find that the

three control samples gave appreciable yields of ether-insoluble bromides (an average polybromide number of 0.427). Calculated as arachidonic acid by the method described previously (19) this would amount to about 0.54 per cent of this acid in the esters. The bromine analysis, 67.12 per cent, was, however, 0.34 per cent too high for methyl octobromoarachidate (66.78), while the average for samples 4, 5, 8, and 9 was 68.14, an increase of 1.02 per cent due to the menhaden oil. The maximum polybromide number

TABLE 2

Analyses of the methyl esters of butter

SAMPLE	IODINE NUMBER	SAPONIFICATION NUMBER	POLYBROMIDE NUMBER	PER CENT BROMINE
1	39.25	207.47	0.385	
2	40.32	204.31	0.442	
3	38.36	205.83	0.453	67.12
Fish oil feeding begins				
4	42.18	205.23	0.651	66.98
5	50.39	206.72	0.586	67.44
6	62.72	205.61	1.765	64.91
8	62.17	207.20	2.590	69.33
9	58.62	207.32	2.047	68.81
10	56.08	205.18	1.416	
Fish oil feeding ends				
12	46.08	204.16	0.645	67.62
14	41.87	205.33	0.595	68.57
Menhaden oil	172.60	187.02	53.5	69.50

was given by sample 9, i.e., 2.59, an increase of about six times due to the fish oil. After ten days of control diet the polybromide number returned nearly to its original value although the bromine analysis was still high.

The appearance of ether-insoluble bromides in samples 1, 2, and 3 indicated the presence in butter of an acid similar to arachidonic. This conclusion was further verified in three specimens of commercial butter. A 5 pound specimen (Fairmont Creamery),

for example, gave methyl esters (iodine number 33.45, saponification number, 208.9) which yielded a polybromide number of 0.24, with 66.97 per cent bromine. These esters contained, therefore, about 0.30 per cent of acid calculated as arachidonic. The question of the identity of this acid was made doubtful, however, by its melting point behavior. Pure methyl octobromoarachidate melts rather sharply at 228° to 230° (uncorrected). The bromides obtained from the butter were compared with a specimen of the pure bromide, and were found to *only partially* melt at 234° to 235° with gas formation which along with the slightly high bromine analysis suggests the presence of more highly unsaturated acids. We have demonstrated, therefore, the presence in normal butter of a fatty acid similar to arachidonic, possibly a mixture of this acid with others more unsaturated. The complete identification of this acid or mixture would require working up of 50 to 100 pounds of butter, and was not further studied. It should be stated here that several other specimens of butter were tested for arachidonic acid with results that were similar but usually slightly higher.

SUMMARY

1. The feeding of menhaden oil to a dairy cow lowered milk production, the percentage of butter fat, and total butter fat. After resumption of control diet recovery to normal took place slowly. There was a tendency toward drying off the animal.

2. The characteristic highly unsaturated acids of the menhaden oil passed into the butter fat in small amounts.

3. The analytical constants of the butter changed to those of a mixture of butter with menhaden oil.

4. Several specimens of normal butter have been shown to contain small quantities (0.3 per cent) of a highly unsaturated fatty acid similar to arachidonic acid.

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THE EFFECT OF DILUTION ON THE TITRATABLE ACIDITY OF COWS' MILK*

H. H. SOMMER AND JULES MÊNOS†

Department of Dairy Husbandry, University of Wisconsin

In determining the titratable acidity of milk various procedures are used. Some titrate the milk directly; others dilute the measured sample with definite amounts of water ranging from 1 to 10 volumes; still others dilute the measured sample with indefinite amounts of water used for rinsing the pipette. If such dilution affects the result as various investigators have reported, then this factor in the "acid test" should not be ignored as is now very commonly the case.

The writers' attention was directed to this subject by several instances. In one case, discrepancies in the titratable acidities reported on the same samples of milk by two different laboratories were traced to the fact that one of the laboratories titrated the milk without dilution, while the other diluted the measured samples with two volumes of water. In another case, several large milk plants frequently referred to their milk as having acidities of 0.10 to 0.12 per cent; and comparing the acidities with those mentioned in neighboring plants (0.15 to 0.17 per cent), the operators had come to the false belief that their milk supply was superior. It was found that they determined the titratable acidity by titrating 9 cc. of milk plus 90 cc. of distilled water. These instances admirably illustrate the unfortunate consequences of ignoring dilution as a factor in the "acid test."

As a consequence of the above observations, the present work was undertaken. The results in the literature seem quite conclusive, but further emphasis of this subject appeared desirable.

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† This article is based on the experimental work of the Master of Science thesis, University of Wisconsin, June, 1930, by the junior author.

TABLE 1

The effect of dilution on the titratable acidity as shown by various investigators

INVESTIGATORS	YEAR	SAMPLE NUMBER	UNDILUTED ACIDITY	DILUTED ACIDITY	DIFFERENCE
(Dilution with 1 volume of water)					
			per cent	*per cent*	*per cent*
Berberich	1907	1	0.1305	0.1192	0.0113
		2	0.1462	0.1205	0.0257
		3	0.1305	0.1125	0.0180
Söldner	1888	1	0.1350	0.1225	0.0125
Siegfeld	1904	1	0.2070	0.1800	0.0270
		2	0.1467	0.1287	0.0180
Hanne	1904	1	0.1233	0.1044	0.0189
		2	0.1467	0.1287	0.0180
(Dilution with 2 volumes of water)					
Berberich	1907	1	0.1305	0.1102	0.0203
		2	0.1462	0.1237	0.0225
		3	0.1305	0.1080	0.0225
		4	0.1520	0.1282	0.0238
Söldner	1888	1	0.1350	0.0990	0.0360
Siegfeld	1904	1	0.2070	0.1630	0.0440
		2	0.1638	0.1260	0.0378
Hanne	1904	1	0.1233	0.0972	0.0261
		2	0.1467	0.1206	0.0261
		3	0.1548	0.1278	0.0270
		4	0.1611	0.1287	0.0324
		5	0.5778	0.5742	0.0026
(Dilution with 3 volumes of water)					
Berberich	1907	1	0.1305	0.0990	0.0315
		2	0.1462	0.1170	0.0292
		3	0.1305	0.1125	0.0180
(Dilution with 4 volumes of water)					
Berberich	1907	1	0.1305	0.0945	0.0360
		2	0.1452	0.1125	0.0327
		3	0.1305	0.0945	0.0360
		4	0.1520	0.1125	0.0395

TABLE 1—*Concluded*

INVESTIGATORS	YEAR	SAMPLE NUMBER	UNDILUTED ACIDITY	DILUTED ACIDITY	DIFFERENCE
(Dilution with 5 volumes of water)					
			per cent	*per cent*	*per cent*
Söldner	1888	1	0.1350	0.0832	0.0518
(Dilution with 10 volumes of water)					
Rice and Markley	1927	3	0.216	0.149	0.067
		1	0.185	0.122	0.063
		26	0.180	0.104	0.076
		2	0.176	0.117	0.059
		4	0.168	0.113	0.055
		15	0.095	0.054	0.041
		18	0.086	0.050	0.036

A further aim of the present study was to offer a more detailed explanation of this dilution effect.

REVIEW OF LITERATURE

That the addition of water to the measured sample of milk lowers the titratable acidity has been shown by Söldner (1), Plaut (2), Hanne (3), Siegfeld (4), Berberich (5), Rice and Markley (6) and others. The results reported by these investigators have been compiled in table 1, and show that dilution lowers the titratable acidity and that this effect becomes progressively more pronounced as the dilution is increased.

It is of interest to note that Berberich studied the effect of dilution on both milk, and milk plus neutral potassium oxalate. In the former, dilution lowered the titratable acidity, but in the latter, dilution was practically without effect.

Several explanations have been offered for the effect of dilution Söldner attributed it to the hydrolysis of the calcium caseinate. Rice and Markley concluded that it is due to the hydrolysis of both the caseinates and phosphates, Barthel (8) explains it through the use of an equilibrium equation:

$$3\ Ca^{++} + 2HPO_4^{--} \rightleftharpoons Ca_3(PO_4)_2 + 2\ H^+$$

stating that dilution shifts the equilibrium in such a manner that the titratable acidity and hydrogen ion concentration are decreased. One of us (9) attributed the effect of dilution to a decrease in the amount of tri-calcium phosphate precipitated during the titration. Thus we may conclude that while there is good agreement among investigators as to the effect of dilution, there is no general accepted explanation of this effect.

EXPERIMENTAL

The magnitude of the dilution effect

In order to demonstrate the effect of dilution, the titratable acidities of a number of fresh milk samples were determined and compared with the acidities found in the same samples after dilution with 1 volume and 9 volumes of distilled water.

The milk samples used were obtained from the University herd; they were taken from individual cows of four different breeds in order to obtain relatively wide differences in composition and in titratable acidity. Precautions were taken to prevent any developed acidity.

In the case of each sample, the following titrations were made with N/10 sodium hydroxide: (1) 10 cc. milk, (2) 10 cc. milk plus 10 cc. distilled water, and (3) 10 cc. milk plus 90 cc. distilled water. A one per cent alcoholic solution of phenolphthalein was used as the indicator; 3 drops were used for the 10 cc. milk, and where water was added the amount of indicator was increased in proportion. The titrations were carried to the first definite, and relatively permanent shade of pink. The cubic centimeters of N/10 sodium hydroxide used were converted into percentage of lactic acid, assuming for convenience that the 10 cc. pipette delivered 10 grams of milk.

The results obtained with 19 different samples of milk are given in table 2. The three titrations in the order given above showed averages of 0.1721, 0.1493, and 0.1098 per cent lactic acid, respectively. These results represent a decrease in titratable acidity of 0.0228 per cent when the milk was diluted with an equal volume of water, and a decrease of 0.0623 per cent when diluted with 9 volumes of water. Fourteen other samples were

titrated undiluted and diluted with an equal volume of water with essentially the same results; the average acidity of the undiluted samples was 0.1831 per cent, as compared with an average of 0.1643 per cent when the same samples were diluted with an equal volume of water. As an average of the 33 samples,

TABLE 2

The effect of dilution on the titratable acidity of milk

NUMBER OF COW	A 10 cc. MILK		B 10 cc. MILK + 10 cc. WATER		C 10 cc. MILK + 90 cc. WATER		DIFFERENCES DUE TO DILUTION	
	N/10 NaOH	Acidity	N/10 NaOH	Acidity	N/10 NaOH	Acidity	A-B	A-C
	cc.	*per cent*	*cc.*	*per cent*	*cc.*	*per cent*	*per cent*	*per cent*
99	1.3	0.117	1.10	0.099	0.70	0.063	0.018	0.054
23x	1.4	0.126	1.10	0.099	0.75	0.067	0.027	0.059
39	1.5	0.145	1.25	0.112	0.95	0.085	0.033	0.060
81	1.5	0.145	1.30	0.117	0.95	0.085	0.028	0.060
6	1.5	0.145	1.35	0.121	1.00	0.090	0.024	0.065
73	1.85	0.166	1.60	0.144	1.20	0.108	0.022	0.058
3y	1.9	0.171	1.70	0.153	1.20	0.108	0.018	0.063
2y	1.9	0.171	1.70	0.153	1.30	0.117	0.018	0.054
1	1.9	0.171	1.70	0.153	1.30	0.117	0.018	0.054
12	1.9	0.171	1.75	0.157	1.30	0.117	0.014	0.054
2x	1.9	0.171	1.65	0.148	1.20	0.108	0.023	0.063
11	1.9	0.171	1.70	0.153	1.25	0.112	0.018	0.059
25	2.0	0.180	1.80	0.162	1.20	0.108	0.018	0.072
12	2.0	0.180	1.75	0.157	1.30	0.117	0.023	0.063
21	2.2	0.198	2.00	0.180	1.40	0.126	0.018	0.072
27	2.2	0.198	1.90	0.171	1.40	0.126	0.027	0.072
14	2.3	0.207	2.00	0.180	1.50	0.135	0.027	0.072
35	2.4	0.212	2.00	0.180	1.60	0.144	0.032	0.068
20	2.5	0.225	2.20	0.198	1.70	0.153	0.027	0.072
Average.....		0.1721		0.1493		0.1098	0.0228	0.0623

dilution with an equal volume of water decreased the titratable acidity 0.0211 per cent. The results obtained are in good agreement with those found in the literature as given in table 1.

Calcium phosphate and the dilution effect

In view of Berberich's finding that dilution of oxalated milk did not affect the titratable acidity, and in view of the explana-

tions for the dilution effect by Söldner, Rice and Markley, Barthel, and one of us, it was decided to study the behavior of solutions containing calcium and phosphates, to determine their behavior during titration, with and without dilution.

As a preliminary experiment, four solutions were prepared and titrated, undiluted and diluted with 9 volumes of water. The solutions were: (1) 2.537 grams mono-calcium phosphate and 1.866 grams calcium chloride per liter, (2) 2.537 grams mono-calcium phosphate per liter, (3) 1.866 grams calcium chloride per liter, and (4) a lactic acid solution, approximately N/5. The results are given in table 3. Solution 1 contained 0.15 per cent

TABLE 3

The effect of dilution on titratable acidity of various solutions

SOLUTION		TITRABLE ACIDITY		
Number	Composition, grams per liter	A 25 cc. undiluted	B Diluted, 25 cc. + 225 cc. H_2O	Difference, A-B
		cc. N/10 NaOH	*cc. N/10 NaOH*	
1	2.537 grams $Ca(H_2PO_4)_2$ + 1.866 grams $CaCl_2$	8.65	7.45	−1.20
2	2.537 grams $Ca(H_2PO_4)_2$	6.30	5.90	−0.40
3	1.866 grams $CaCl_2$	0.10	0.95	+0.85
4	Approximately N/5 lactic acid	46.20	47.10	+0.90

CaO and 0.15 per cent P_2O_5 and is therefore fairly representative of the calcium and phosphate content of milk. Solutions 2 and 3 respectively represent the amounts of mono-calcium phosphate and calcium chloride required to make Solution 1, but dissolved separately. Solution 4, lactic acid, was included as a matter of interest.

In the case of Solutions 3 and 4, dilution increased the titratable acidity; this result can readily be explained on the basis of hydrolysis. Solution 2, mono-calcium phosphate, showed a decrease in acidity on dilution; this might conceivably also be explained on the basis of hydrolysis, but it should be recorded that precipitation was observed during the titration, and the amount of precipitate was apparently less in the diluted sample.

A significant indication is gained from a comparison of Solutions 1, 2, and 3, titrated undiluted. Solution 2 had a titer of 6.3 cc., and Solution 3 had a titer of 0.1 cc.; yet when the two were combined as in Solution 1, a titer of 8.65 cc. N/10 NaOH was obtained. Similarly these solutions, titrated after dilution with 9 volumes of water, give a significant indication. Solution 3, calcium chloride, had an increased titer on dilution, yet when this amount of calcium chloride was added to Solution 2 to produce Solution 1, a greater decrease in titer on dilution resulted (compare the decrease in titer due to dilution in Solutions 1 and 2). As will be discussed more fully later, these results can be explained only on the basis of tri-calcium phosphate precipitation.

In order to demonstrate the behavior of solutions containing calcium and phosphates more clearly, electrometric titrations of Solution 1 were undertaken. In the preliminary electrometric titrations it was found that precipitation of tri-calcium phosphate occurred, that the precipitation was quite slow and that there is a decrease in the pH of the solution as precipitation progresses. Wendt and Clarke (10), and Holt, LaMer, and Chown (11) have reported a similar observation; in electrometric titration of similar solutions these investigators found it necessary, after each addition of alkali, to wait several days before making the pH reading, if consistent results were to be obtained.

As a result of the above, the following procedure was adopted: A series of samples was prepared by placing 25 cc. of Solution 1 into each of 25 screw cap jars, and adding accurately measured amounts of N/10 NaOH in such a manner as to produce a series of samples with successively larger additions of NaOH from 0 to 10 cc. A similar series of samples was prepared but containing 25 cc. of Solution 1 and 225 cc. of distilled water. The jars, securely closed to prevent evaporation, were allowed to stand about 12 hours; from each jar a portion of the clear solution was then pipetted off, taking care not to include any precipitate, and used for the pH determinations. The precipitate, in those cases where any had formed, was collected by filtering through a weighed Gooch crucible, using the clear filtrate to complete the transfer from the jar. The crucibles were then dried to constant

weight. The results are given in figure 1, showing both the electrometric titration curves and the amounts of precipitate.

It will be noted in figure 1 that there is a break in each of the titration curves, a decrease in pH in spite of an increase in the

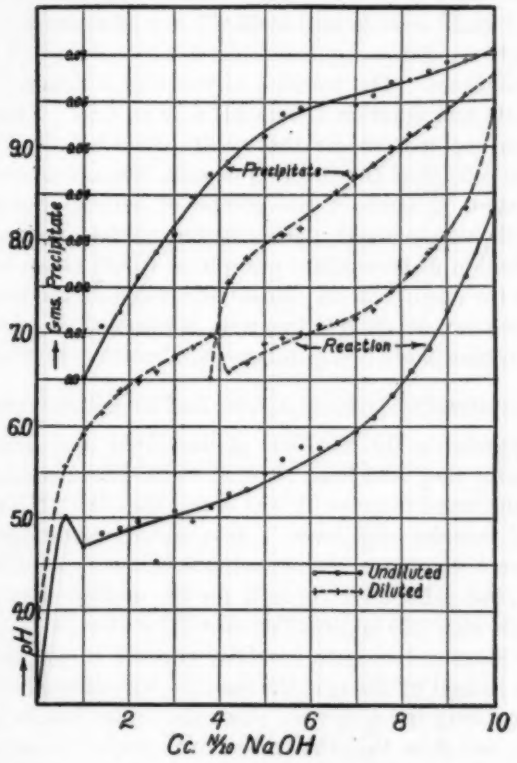

FIG. 1. TITRATION AND PRECIPITATION CURVES OF A MONO-CALCIUM PHOSPHATE-CALCIUM CHLORIDE SOLUTION TITRATED UNDILUTED AND DILUTED WITH NINE VOLUMES OF WATER

amount of sodium hydroxide added, and that this break coincides with the occurrence of the precipitate. Further, the break occurs much earlier in the undiluted mono-calcium phosphate-calcium

chloride solution than in the solution diluted with 9 volumes of water. It is clear that the precipitation of tri-calcium phosphate plays a significant rôle in the titration of such solutions.

Although not strictly related to the present subject, it is of interest to note here that when freshly prepared portions of Solutions 1 and 2 were heated at 62.8°C. for 30 minutes, Solution 2 showed only a slight precipitate while Solution 1 showed an appreciable precipitate. The reaction of Solution 2 changed from pH 6.64 to 5.18, and Solution 1 from pH 6.72 to 4.85. These results suggest an explanation for the results observed by Van Slyke and Baker (12) that the reaction of milk remains unchanged by pasteurization in spite of the escape of carbon dioxide, while in carbon dioxide free milk, pasteurization causes a decrease in pH. A precipitation of tri-calcium phosphate would cause a decrease in pH but the loss of carbon dioxide from the milk has the opposite effect; when carbon dioxide free milk is pasteurized, the effect of tri-calcium phosphate precipitation then becomes evident.

The phenolphthalein end-point and the dilution effect

On determining the reaction of undiluted and diluted milk samples after they had been titrated to the phenolphthalein end-point in the usual manner, it was found that the pH in the case of diluted samples was lower. As a result, a number of milk samples were titrated to the phenolphthalein end-point as already described, but using 25 cc. of milk for the undiluted samples and 25 cc. milk plus 225 cc. distilled water for the diluted samples. After the samples had been carefully titrated to the same shade of pink as judged by the eye, the reaction was determined electrometrically, using the hydrogen electrode. The results are given in table 4, and show that the end-point is reached at an appreciably lower pH in the diluted samples.

The reason for the above differences in reaction at the end-point in undiluted and diluted milk was sought in the effect of salts and proteins on the phenolphthalein; dilution would reduce the "salt and protein errors." The reaction at the phenolphthalein end-point was determined in four samples of milk, titrated, diluted with 9 volumes of water, and diluted with 9 volumes of a 0.7 per

TABLE 4

The reaction at the phenolphthalein endpoint in undiluted and diluted milk samples

UNDILUTED SAMPLES		DILUTED SAMPLES, 9 VOLUMES OF WATER ADDED		DIFFERENCE, pH
Cc. N/10 NaOH	Phenolphthalein endpoint, pH	Cc. N/10 NaOH	Phenolphthalein endpoint, pH	
5.2	8.59	4.2	7.72	0.87
5.2	8.48	4.4	7.97	0.51
5.3	8.61	4.3	7.94	0.67
5.5	8.46	4.9	8.30	0.16
5.6	8.63	5.0	8.24	0.39
5.25	8.34	3.9	8.22	0.12
5.6	8.39	3.9	8.16	0.23
5.85	8.73	4.7	8.15	0.58
6.05	8.49	4.0	8.01	0.48
6.0	8.39	4.0	8.16	0.23
6.2	8.42	4.8	8.07	0.35
6.45	8.67	4.5	7.97	0.70

TABLE 5

Effect of casein on the phenolphthalein endpoint

COMPARISON NUMBER	UNDILUTED CASEIN SOLUTIONS, PHENOLPHTHALEIN ENDPOINT, PH	CASEIN SOLUTIONS + 9 VOLUMES OF WATER, PHENOLPHTHALEIN ENDPOINT, pH	DIFFERENCE
1	8.30	8.10	0.20
2	8.49	8.32	0.17
3	8.51	8.25	0.26
4	8.62	8.34	0.28
5	8.49	8.30	0.19
6	8.62	8.34	0.28
7	8.73	8.19	0.54
8	8.63	8.04	0.59
9	8.57	8.15	0.42
10	8.47	8.25	0.22
11	8.52	8.24	0.28
12	8.50	8.32	0.18
13	8.69	8.35	0.34

cent common salt solution. The samples diluted with water had reactions at the phenolphthalein end-point as follows: pH 8.01, 8.16, 8.16, and 8.22. The same samples diluted with 0.7 per cent common salt solution had reactions as follows: pH 7.92, 7.94,

7.93, and 8.06. Thus at the higher salt concentration, the end-point is reached at a slightly lower pH, than diluted milk. The effect of casein concentration on the end-point was observed by titrating casein solutions, undiluted and diluted with 9 volumes of water, and determining the reaction at the end-point. The casein solution used, was made by dissolving 4 grams of purified casein in 100 cc. lime water, dialyzing 24 hours, and neutralizing to pH 6.60 with N/10 HCl–M/20 sodium citrate solution. The undiluted and diluted casein solutions were titrated at the same time in each comparison in order to match the end-points as closely as possible. Thirteen such comparisons were made, and in each case the end-point in the diluted casein solution was reached at a lower pH. The results are given in table 5. The effect of casein concentration on the end-point of the indicator accounts for the greater part of the difference in the pH at the end-point in undiluted and diluted milk.

The above experiments should be regarded as preliminary; more experiments on the effect of other milk salts and proteins on the phenolphthalein end-point, are desirable, but time did not permit their undertaking.

DISCUSSION

The results on the effect of dilution on the titratable acidity of milk, as found in this work, (table 2) are in good agreement with those reported in the literature (table 1). Dilution of the measured sample of milk with an equal volume of distilled water was found to lower the titratable acidity 0.211 per cent; dilution with 9 volumes of water lowered it 0.0623 per cent.

An important factor in this effect of dilution is the decreased precipitation of tri-calcium phosphate during the titration. A solution representative of milk with respect to calcium and phosphate content (Solution 1, table 3) also showed a decreased titer on dilution with 9 volumes of water; the decrease in titer was equivalent to 0.043 per cent lactic acid. That this decreased titer is due to less tri-calcium phosphate precipitation is indicated by the results given in figure 1.

The manner in which the precipitation of tri-calcium phosphate

affects the titratable acidity is indicated more clearly by figure 2, reproduced from the work of Holt, LaMer and Chown. Figure 2 gives the titration curves of phosphoric acid, titrated with sodium hydroxide and calcium hydroxide. It will be noted that in the titration with sodium hydroxide, the first equivalent of phosphoric acid has been neutralized at about pH 4.5, and the second at pH 8.8. This well known titration curve leads to the common

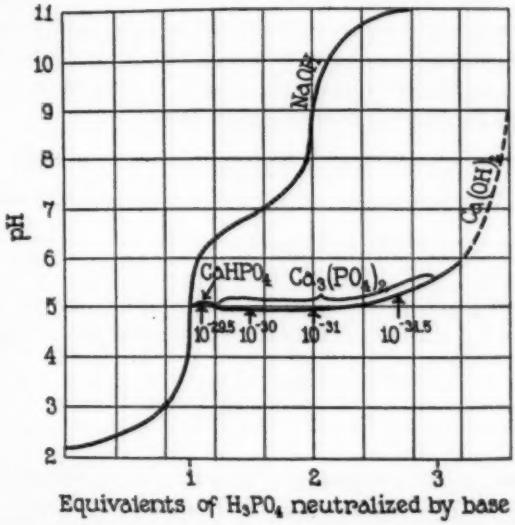

FIG. 2. TITRATION CURVES OF PHOSPHORIC ACID TITRATED WITH SODIUM HYDROXIDE AND CALCIUM HYDROXIDE
(Reproduced from Holt, LaMer and Chown)

statement that in the titration of phosphoric acid or acid phosphates, we have titrated only to the di-basic salt at the phenolphthalein end-point. However, in the titration with calcium hydroxide, it will be seen from figure 2 that all three of the acid hydrogens of the phosphoric acid have been titrated long before the pH of the phenolphthalein end-point has been reached. This can only occur only because of the precipitation of tri-calcium phosphate.

The precipitation of tri-calcium phosphate can occur only if there are tertiary phosphate ions present. It is a common error to say that below pH 4.5 there are no secondary phosphate ions, and below pH 8.8 there are no tertiary phosphate ions present in the solution (see fig. 2). The presence of tertiary phosphate ions below pH 8.8 must necessarily obtain as shown by calculations from the dissociation constants of phosphoric acid. It is true that the tertiary phosphate ion concentration may be insignificant and can be ignored for such purposes as calculating buffer mixtures, but when the progressive removal of tertiary phosphate ions by precipitation is involved, even a very small concentration of such ions becomes of utmost significance.

TABLE 6

Calculated tertiary phosphate ion and calcium ion concentrations in milk at various reactions

pH	(H^+)	(PO_4^{---})	(Ca^{++})	$(Ca^{++})^3 \times (PO_4^{---})^2$
6.5	3.12×10^{-7}	6.554×10^{-9}	1.07×10^{-2}	5.304×10^{-22}
7.0	1×10^{-7}	3.514×10^{-8}	1.07×10^{-2}	1.515×10^{-21}
7.5	3.12×10^{-8}	1.477×10^{-7}	1.07×10^{-2}	2.694×10^{-20}
8.0	1×10^{-8}	5.058×10^{-7}	1.07×10^{-2}	3.159×10^{-19}
8.5	3.12×10^{-8}	1.673×10^{-6}	1.07×10^{-2}	3.157×10^{-18}

To illustrate the above and to give us a more definite basis for a discussion of the dilution effect, the calculations given in table 6 have been made. In these calculations it has been assumed that the P_2O_5 content of milk is 0.15 per cent and that the phosphates are 70 per cent dissociated, and that the calcium oxide content is 0.15 per cent and that only 40 per cent is diffusible and dissociated. Thus the total phosphate ion concentration is assumed to be 1.50/71 $\times$ 0.70 or 0.01477 molar, and the calcium ion concentration is assumed to be 1.50/56 $\times$ 0.40 or 0.0107 molar. The tertiary phosphate ion concentration was calculated from the dissociation constants, k_2 and k_3, of phosphoric acid.

The calculations involved are illustrated by the following calculation for pH 6.5 ($H^+ = 3.12 \times 10^{-7}$). From k_2 (1.95×10^{-7})

we know that the ratio of primary to secondary phosphate ion concentrations must be:

$$\frac{(H^+)}{1.95 \times 10^{-7}} = \frac{(H_2PO_4^-)}{(HPO_4^{--})} = \frac{3.12 \times 10^{-7}}{1.95 \times 10^{-7}}$$

Knowing the ratio of primary to secondary phosphate ion concentrations, and having assumed that the total phosphate ion concentration is 0.01477, we can now compute the secondary phosphate ion concentration. In this step of the calculation we can ignore the tertiary phosphate ion concentration without introducing a significant error. If the total phosphate ion concentration is 0.01477 and if there are 3.12 parts of primary to 1.95 parts of secondary phosphate, then the secondary phosphate ion concentration will be:

$$\frac{1.95}{3.12 + 1.95} \times 0.01477 = 0.00568$$

From this figure and from k_3 (3.6×10^{-13}) we can now calculate the tertiary phosphate ion concentration at pH 6.5, as follows:

$$\frac{(H^+)}{3.6 \times 10^{-13}} = \frac{(HPO_4^{--})}{(PO_4^{---})}$$

Substituting 3.12×10^{-7} for (H^+), and 0.00568 for (HPO_4^{--}) and calculating for (PO_4^{---}), we get $(PO_4^{---}) = 6.554 \times 10^{-9}$. Similarly the concentrations of tertiary phosphate ions may be calculated for the other reactions shown in the table.

The solubility product of tri-calcium phosphate has been investigated by Holt, LaMer and Chown (11) and by Sendroy and Hastings (13) in connection with blood serum at 38°C. They found that the solubility product is influenced by the presence of other salts; values have been reported ranging from 1×10^{-26} to $1 \times 10^{-32.5}$. We are not justified in applying these values to milk for exact calculations but to demonstrate in a qualitative sense what we may expect in milk is justified. For such a purpose we may take $(Ca^{++})^3 \times (PO_4^{---})^2 = 1 \times 10^{-26}$, as the solubility product of tri-calcium phosphate. Compare the figures in the last column of table 6 with this value; it will be noted that

even at pH 6.5, the calculated $(Ca^{++})^3 \times (PO_4^{---})^2$ value exceeds the above solubility product, and that with increases in the pH, the calculated values become still greater. The conclusion is that precipitation of tri-calcium phosphate must be expected during the titration of milk.

The precipitation of tri-calcium phosphate increases the titratable acidity, because, by the removal of tertiary phosphate ions by precipitation, the equilibrium equation,

$$HPO_4^{--} \rightleftharpoons H^+ + PO_4^{---}$$

proceeds to the right; the result is that the third acid hydrogen must be neutralized to the extent that the tertiary phosphate ions are removed by precipitation.

If the milk is diluted to ten times its original volume by the addition of 9 volumes of distilled water, the tertiary phosphate ion and calcium ion concentrations will be lowered to approximately one-tenth of their former value. Precipitation will therefore occur later in the titration and will be considerably less at the phenolphthalein end-point. The result is a lower titratable acidity.

The calculations on which the above conclusion is based, involve assumptions as to the calcium ion concentration, the tertiary phosphate ion concentration, and the solubility product of tri-calcium phosphate. These assumptions might be varied appreciably without impairing the validity of the above conclusion. For example, even if we assume that the calcium ion concentration of milk is only 1 per cent of the total calcium content, or $(Ca^+) = 2.8 \times 10^{-4}$, as Whittier (15) concluded on the basis of calculations, the above calculations will still indicate precipitation of tri-calcium phosphate during the titration to the phenolphthalein end-point. The calculations given above are intended only for the purpose of making the discussion concrete; the object of the discussion is to point out the qualitative facts that precipitation of tri-calcium phosphate is favored as the pH is increased, and that less precipitation of tri-calcium phosphate takes place in more dilute solutions, and to indicate that these facts are involved in the titration of milk.

While this work does not include any experimental results on the effect of casein in the presence of calcium, there are indications that the acidity contributed to the milk by the casein is influenced by the calcium content in a manner similar to the phosphates. Casein acts as a weak polybasic acid, the successive dissociations of which are favored by increases in pH. As a result, casein is capable of combining with additional bases as the neutralization progresses, and thus it contributes to the titratable acidity of milk as Rice and Markley (6) have shown. However, if there is a difference in the solubility of the caseinates of various bases, then the titratable acidity contributed by the casein may be affected by the bases present in a manner similar to the phosphates.

That there is a difference in the solubility of calcium caseinate and sodium caseinate is indicated by the fact that in the presence of both bases, the calcium caseinate is formed. Further evidence is furnished by the work of Osborne (7) who found that calcium caseinate did not filter through a Pukall filter, but on the addition of even small amounts of ammonium or potassium oxalate to such a caseinate solution, the filtrate showed the presence of casein; when sufficient oxalate was added and the solution allowed to stand for several days, the filtrate contained all of the casein.

If we regard the calcium caseinates as insoluble, then we must expect that the caseinates will behave in a manner entirely analogous to the phosphates. Porcher (14) has performed experiments which bear quite directly on this subject; he found as follows:

AMOUNTS OF $CaCl_2$ ADDED	ADDED TO 1 LITER DISTILLED WATER	ADDED TO 1 LITER Ca CASEINATE SOLUTION
grams	*pH*	*pH*
0.0	5.08	6.62
1.0	6.08	6.42
2.0	6.58	6.49

The addition of calcium chloride to water increased the pH, while the addition of the same amount of calcium chloride to calcium caseinate caused a decrease in pH. A possible explanation of this result is that the addition of the calcium ions caused the formation of less soluble calcium caseinates with a resulting

further dissociation of the casein in a manner analogous to the behavior of the phosphates.

As has already been pointed out, Berberich's work showed that the dilution effect involves the calcium salts; the titratable acidity of oxalated milk was not affected by dilution. Berberich also found that rather large additions of oxalate are required before the fullest effect of such additions are realized. These facts suggest that in addition to phosphates, there is some other substance, probably casein, involved with the calcium in the titration of oxalated milk and in the dilution effect. If only calcium in relation to phosphates were involved, it would seem that a relatively small addition of oxalate should suffice; because of the rather low solubility product of calcium oxalate and the low concentration of tertiary phosphate ions, large additions of oxalate should not be necessary to prevent the precipitation of tricalcium phosphate. These considerations lend support to the belief that a calcium to casein relationship is involved in the dilution effect. Further experimental evidence on this point would be desirable.

Another factor responsible for the lower titratable acidity of diluted milk samples is the observed fact that the pH at the phenolphthalein end-point is always considerably lower in the diluted samples. Not enough work was done to determine exactly the cause for this observation, but indications are that the main cause is the effect of the proteins on the end-point of the indicator. A high protein concentration shifts the end-point to a higher pH; diluting therefore lowers the pH at the end-point.

Another factor that has been suggested in explanation of the lower titratable acidity of diluted milk samples is the increase in pH when milk is diluted with water. The thought apparently is that since dilution of the milk increases the pH slightly, not as much alkali will be required in the titration because the reaction is slightly closer to the phenolphthalein end-point to begin with. This logic is fallacious as can be demonstrated on the basis of theoretical considerations; it is sufficient here to point out that dilution of an approximately N/5 lactic acid solution (as in table 3) certainly increases the pH, but the titratable acidity is increased.

While the main object of this study was to determine and explain the effect of dilution on the titratable acidity of milk, the facts revealed by the experiments enable us to explain other phenomena, viz. (*a*) the fading of the end-point in titrating milk, (*b*) the titratable acidity of condensed milk, and (*c*) the neutralization with lime.

It is a common experience in titrating milk to observe that the pink color of the phenolphthalein end-point fades quite rapidly. This is commonly explained by saying that carbon dioxide is absorbed from the air, but the inadequacy of this explanation is indicated by the fact that other solutions, which are quite as good solvents for carbon dioxide, are not subject to such rapid fading. A more logical explanation for the rapid fading is based on tri-calcium phosphate precipitation. Results given here and in the literature show that milk or a phosphate solution will become more acid (lower pH) as a result of phosphate precipitation. The results of Wendt and Clarke (10), verified in the present study, show that several hours are required for this precipitation to reach equilibrium. Hence the explanation for the fading is as follows: When the milk has been brought to the pink color, the precipitation of the tri-calcium phosphate has not reached equilibrium, further precipitation occurs within a comparatively short time and the resulting drop in pH explains the fading.

The titratable acidity of concentrated milk is usually higher than calculated from the ratio of concentration and the acidity of the original milk. This is true in spite of the fact that carbon dioxide is known to have been expelled in the condensing process. This observation is to be explained by the same factors involved in the dilution effect, but in this case we are dealing with an increased concentration and these factors therefore cause an increased titratable acidity.

In the neutralization of milk or cream with lime of definitely known alkalinity, it is common to note that the acidity attained is not as low as calculated. This is due to the fact that the addition of lime increased the calcium ion concentration with the result that in subsequent titrations there is more tri-calcium

phosphate precipitated and hence a higher titratable acidity is obtained than was calculated from the original titration and the known amount of lime added. It is for a similar reason that titrating milk with standard barium hydroxide solution leads to higher titratable acidities than titration with sodium hydroxide, as shown by Siegfeld (4), and Plaut (2).

SUMMARY AND CONCLUSIONS

1. Dilution of the measured sample of milk lowers the titratable acidity; dilution with an equal volume of water caused an average decrease of 0.0211 per cent, and dilution with 9 volumes of water, an average decrease of 0.0623 per cent.

2. It has been demonstrated that in solutions containing calcium and phosphates in amounts representative of milk, precipitation of tri-calcium phosphate will occur during titration with sodium hydroxide, and that such precipitation increases the titer. By calculation it has been shown that a similar precipitation must be expected in the titration of milk.

3. The greater part of the dilution effect is attributed to a decrease in the amount of tri-calcium phosphate precipitated during the titration.

4. There are indications that a relationship of calcium to casein, similar to that of calcium to phosphates, is involved in the dilution effect.

5. A lower pH was observed at the phenolphthalein end-point in the titration of diluted milk. This is attributed to a "protein effect" on the indicator.

6. The rapid fading of the end-point in titrating milk is attributed to the precipitation of tri-calcium phosphate.

7. The fact that the titratable acidity of concentrated milk is higher than calculated from the acidity of the original milk and the ratio of concentration, in spite of the loss of carbon dioxide, is attributed to the reverse of the same factors involved in the dilution effect.

8. The neutralization of milk or cream with lime of known alkalinity does not lower the acidity to the calculated point because the increased calcium ion concentration causes greater precipitation of tri-calcium phosphate in subsequent titration.

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AGGLUTININ, COMPLEMENT, AND CHEMICAL COMPOSITION OF THE COLOSTRUM OF THE COW*

M. SATO, K. OGURA AND H. IKEJIMA

Zootechnical Institute, Hokkaido Imperial University, Sapporo, Japan

INTRODUCTION

That the colostrum contains a large number of antibodies which are transmitted to new-born cattle and thus play a very important part in assuring the healthful growth of the latter is a well known fact, Bertarelli (1), Famulener (2), Zubrzycki und Wolfsgruber (3), Reymann (4) Little and Orcutt (5). The works of eminent scholars agree in the opinion that antibodies are most abundant in the colostrum obtained immediately after parturition and afterwards they speedily decrease and finally become extinct. What is interesting to state is that although the presence of any antibody in the blood of new-born calves immediately after parturition has never been proved, the colostrum, when first given them, is easily transmitted through the digestive organ without causing any abnormal phenomenon whatever. Consequently it may be asserted that the new-born calves, when fed with the colostrum, will be immune to a variety of organisms. The results of experiments by Smith (8) and others show that sucking calves not fed with the colostrum are easily infected by certain bacteria, particularly *Bacterium coli*, and are apt to die.

Besides antibodies, the presence of complements has been reported in the colostrum (also in the milk of cow suffering from mastitis, Bauer and Sassenhagen), the complement being secreted for a period of over ten days after parturition. The presence of complement is essential for the normal operation of certain antibodies. For instance, bacteriolysin requires the presence of complement for the operation of its bactericidal action. For this reason the presence of complement in the colostrum may be of unusual significance.

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The milk secreting organ of a milch cow may easily be affected by diverse circumstances producing abnormal conditions. Consequently the percentage of milch cows secreting normal colostra from physical, chemical, and physiological standpoint may be very small. Experiments conducted by one of the authors for upwards of 10 years with colostra obtained from a large number of cows has indicated the prevalence of abnormal colostra, an observation which may explain the variety of previously published investigations concerning colostra. In these cases, all researches and investigations dealing with the antibody content of colostrum will be of little significance unless the so-called normal colostrum is used in the experiments.

The authors have been and are conducting the researches concerning the immunity given by the colostra since January, 1925. The following is a report of the results of chemical analyses, and of the agglutination tests and the investigations as to the presence of complement which they carried out up to March, 1926, with normal colostra obtained from 16 cows of Ayrshire, Guernsey, and Holstein breeds and one sample of milk from a cow infected with mastitis and another after abortion.

PARTICULARS CONCERNING THE COWS

The colostrum used for the experiment was obtained from the cattle of Ayrshire breed kept at the first and from Holsteins and Guernseys kept at the second farms of the Faculty of Agriculture, Hokkaido Imperial University.

The particulars are given in table 1.

PHYSICAL AND CHEMICAL ANALYSIS OF THE COLOSTRA

Throughout the experiments, the color was determined with J. W. Lovibond's tintmeter and the measurement of hydrogen ion concentration by the potentimeter. In the alcohol test, a certain quantity of the colostrum mixed with equal quantity of alcohol (68 per cent) was put into a test tube, the whole contents being slightly shaken. In the test, the degree of coagulation was examined by putting the test tube containing the colostrum into boiling water. The determination of specific gravity exceeding

TABLE 1

Information on the history of the cows used for the experiments

EXPERIMENT NUMBER	BREED	DATE OF BIRTH	MAXIMUM DAILY MILK PRODUCTION FOR EACH LACTATION PERIOD PRIOR TO EXPERIMENTS								
			1	2	3	4	5	6	7	8	9
			pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
1	Ayrshire No. 94	April, 1918	46.5	46.5	43.0						
2	Ayrshire No. 94					30.8					
3	Ayrshire No. 96	June 28, 1921			45.0	42.0	42.0				
4	Ayrshire No. 115	May 28, 1921									
5	Ayrshire No. 110	June 28, 1921	30.5								
6	Ayrshire No. 116	May 1, 1923									
7	Guernsey No. 173	November 30, 1918	25.2	27.7	31.0	34.7	27.9				
8	Guernsey No. 168	September 22, 1916	23.3	18.0	31.4	32.9	40.4	39.7	14.2		
9	Guernsey No. 189	August 9, 1917	18.3								
10	Holstein No. 195	August 9, 1917	37.8	62.9	58.8	59.9	72.9				
11	Holstein No. 237	April 12, 1923									
12	Holstein No. 171	March 10, 1915	39.0	53.5	56.3	60.7	69.5	29.5	80.7		
13	Holstein No. 243	July 28, 1923									
14	Holstein No. 197	November 8, 1917	36.7	50.3	70.4	83.3	77.0				
15	Holstein No. 173	June 28, 1915	35.4	44.5	32.5	35.8	58.5	55.9	48.3		
16	Holstein No. 239	June 23, 1923									
17	Guernsey No. 170	Feb. 15, 1917	24.4	26.5	33.8	46.8	27.8	*			
18	Holstein No. 169	March 2, 1915	42.2	54.0	55.8	59.3	66.1	87.2	37.3	98.0	†

* Inflammation of under following parturition.

† Samples taken after an abortion on the 110th day after conception and 220 days after previous parturition.

1.04 was made by the use of a Westphal balance at the temperature of 15°C., Quevenne-Müller's lactometer being employed in the determination of specific gravity below 1.04, the degrees ascertained being converted to 15°C. In the case of the colostrum secreted immediately after parturition which was too thick and cohesive for the Westphal balance, a pycnometer was used. The determination of total solids, total protein, fat, ash, casein, and a portion of albumin were made according to the ordinary methods. The results of the experiment are shown in table 2.

AGGLUTINATION TEST

Milk serum. Fresh colostrum was coagulated by mixing with such a quantity of rennet solution (rennet solution, pH 5.69, prepared at Hansen's Laboratory, N. Y., U S. A.) that would coagulate the mixture at 40°C. in approximately 40 minutes. After filtration the clear filtrate was used in the tests.

Bacterial sus pension. Two milligrams of a 2 hour growth was mixed with 1 cc. of physiological saline. This suspension was preserved for use as antigen with 1 per cent formalin.

The milk serum was heated for about 30 minutes at 56°C. and after being made inactive the liquid was diluted with physiological salt solution. To this liquid was added an equal quantity of bacterial suspension, the mixture being completely mixed and incubated for 2 hours at 37°C. The liquid was afterward left at room temperature for 22 hours, after which the agglutination-titre was observed. The results of this experiment are shown in table 3.

Demonstration of complement

The experiment was made by means of a horse erythrocyte-rabbit hemolytic system using as complement fresh colostrum from which the greater portion of fat was removed by means of a centrifugal separator, the time required being about 10 minutes and the number of revolutions 3000 per minute.

Suspension of horse erythrocytes. A 1 per cent suspension of washed (three times) horse erythrocytes was used. Amboceptor used in the demonstration process consisted of the blood of rabbits

TABLE 2

The chemical composition of colostra

NUMBER OF EXPERIMENT	NUMBER OF PARTURITION	HOURS AFTER PARTURITION	NUMBER OF MILKING	COLOR			pH	ALCOHOL TEST	BOILING TEST	SPECIFIC GRAVITY	TOTAL SOLID	TOTAL PROTEIN	CASEIN	LACTO-GLOBULIN	ALBUMIN	FAT	LACTOSE	ASH	MILK QUANTITY
				Red	Yellow	Blue					per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	pounds
I	4	Immediately after	1	1.5	1.5	0	6.03	+	+	1.071	28.51	18.44	—	—	—	7.37	1.51	1.19	11.0
		4	2	1.4	1.4	0	6.05	+	+	1.057	27.98	16.38	—	—	—	8.48	2.04	1.08	4.0
		12	3	1.3	1.2	0	6.08	+	+	1.053	23.98	11.25	—	—	—	9.51	2.15	1.07	3.5
		24	4	1.0	0.8	0	6.08	+	−	1.042	14.60	8.17	—	—	—	2.30	3.17	0.96	14.0
		29	5	1.1	1.0	0	5.92	+	"	1.027	24.85	8.08	—	—	—	13.12	2.80	0.85	4.5
		34	6	1.0	0.7	0	5.91	+	"	1.036	16.66	6.50	—	—	—	5.86	3.43	0.87	6.5
		48	7	0.6	0.5	0.7	5.74	+	"	1.038	14.81	6.59	—	—	—	3.84	3.56	0.82	16.5
		53	8	0.6	0.5	0.7	6.13	+	"	1.036	19.23	7.29	—	—	—	7.72	3.33	0.89	6.0
		58	9	0.6	0.5	0.7	6.14	+	"	1.036	14.66	5.91	—	—	—	4.44	3.54	0.77	4.0
		72	10	0.6	0.5	0.7	6.03	+	"	1.032	14.25	6.52	—	—	—	3.41	3.61	0.71	17.5
		77	11	0.6	0.5	0.7	6.11	−	"	1.029	19.93	5.91	—	—	—	9.98	3.27	0.77	5.8
II	5	19 hours before		1.1	1.0	0	6.09	+	+	1.076	25.96	19.36	13.98		5.38	3.52	2.11	0.97	12.0
		Immediately after	1	1.0	1.2	0	6.04	+	+	1.068	30.00	17.29	8.41		8.88	9.05	2.45	1.21	2.8
		9	2	1.0	0.9	0	—	+	+	1.046	16.02	4.99	4.22		0.77	6.98	3.19	0.86	2.6
		33	3	—	—	—	6.24	+	−	1.037	13.94	4.67	4.10		0.57	6.01	2.48	0.78	11.8
		48	4	—	—	—	6.35	+	"	1.0375	14.43	3.54	3.09		0.45	5.97	3.83	0.79	6.0
		51	5	—	—	—	—	+	"	1.0343	14.84	4.23	4.01		0.32	5.52	4.23	0.76	4.5
		57	6	—	—	—	6.25	+	"	1.0337	—	—	—		—	—	—	—	16.0
		62	7	—	—	—	6.40	+	"	1.037	—	—	—		—	—	—	—	7.0

		76	8	—	—	—	—	+	"	1.0334	—	—	—	—	—	—	—	4.5
		81	9	—	—	—	6.19	+	"	1.034	—	—	—	—	—	—	—	18.5
II	5	105	10	—	—	—	6.23	+	"	1.038	—	—	—	—	—	—	—	7.5
		110	11	—	—	—	—	−	"	—	—	—	—	—	—	—	—	4.9
		18	3	0.4	0.4	0	6.38	+	−	1.051	18.48	10.14	4.04	6.10	5.47	1.81	1.06	3.5
		33	4	0.4	0.3	0.4	6.26	+	"	1.041	—	—	—	—	—	—	—	5.8
		37	5	0.4	0.4	0.4	6.11	+	"	1.0365	15.02	5.65	3.97	1.68	4.34	4.13	0.90	4.0
		57	6	0.1	0.1	0.4	6.24	+	"	1.034	—	—	—	—	—	—	—	13.7
		61	7	0.1	0.1	0.4	6.45	+	"	1.032	15.88	4.98	4.47	0.51	6.38	3.66	0.86	6.5
III	6	66	8	0.1	0.1	0.5	—	+	"	1.034	—	—	—	—	—	—	—	5.0
		81	9	0.2	0.1	0.5	—	−	"	1.036	—	—	—	—	—	—	—	16.2
		85	10	0.2	0.2	0.4	—	"	"	1.032	14.58	4.46	3.68	0.78	5.27	4.11	0.74	7.1
		90	11	0.1	0.1	0.4	—	"	"	1.033	—	—	—	—	—	—	—	5.8
		105	12	—	—	—	—	"	"	1.032	—	—	—	—	—	—	—	20.3
		109	13	—	—	—	—	"	"	1.034	—	—	—	—	—	—	—	7.6
		12	3	0.4	0.4	0	6.45	+	−	1.046	22.78	9.96	7.22	3.74	—	—	1.04	1.6
		39	4	0.4	0.2	0.4	6.52	+	"	1.039	16.04	4.19	3.29	0.90	—	—	0.90	2.8
		43	5	0.5	0.4	0.4	6.46	+	"	1.035	17.06	3.67	3.05	0.62	—	—	0.81	1.0
		63	6	0.3	0.1	0.3	6.51	+	"	1.034	—	—	—	—	—	—	—	6.8
		67	7	0.4	0.1	0.3	6.51	+	"	1.033	—	—	—	—	—	—	—	2.8
		72	8	0.4	0.1	0.3	6.52	+	"	1.033	—	—	—	—	—	—	—	3.0
IV	1	76	9	0.2	0.2	0.3	—	+	"	1.036	11.70	3.83	3.15	0.68	—	—	0.77	8.4
		96	10	0.3	0.1	0.4	—	+	"	1.034	—	—	—	—	—	—	—	3.2
		100	11	0.3	0.1	0.5	—	+	"	1.0335	—	—	—	—	—	—	—	3.5
		115	12	0.4	0.2	0.5	—	−	"	1.033	—	—	—	—	—	—	—	12.0
		120	13	0.3	0.1	0.7	—	"	"	1.033	13.72	3.92	3.43	0.49	—	—	0.88	4.7
		130	14	0.3	0.2	0.9	—	"	"	1.035	—	—	—	—	—	—	—	3.6

TABLE 2—*Continued*

NUMBER OF EXPERIMENT	NUMBER OF PARTURITION	HOURS AFTER PARTURITION	NUMBER OF MILKING	COLOR			pH	ALCOHOL TEST	BOILING TEST	SPECIFIC GRAVITY	TOTAL SOLID	TOTAL PROTEIN	CASEIN	LACTO-GLOBULIN	ALBUMIN	FAT	LACTOSE	ASH	MILK QUANTITY
				Red	Yellow	Blue													
											per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*pounds*
V	2	Immediately after	1	1.1	1.5	0	—	+	+	1.061	—	—		—	—	—	—	—	11.0
		4	1	0.6	0.6	0.4	—	+	+	1.0525	—	—		—	—	—	—	—	7.3
		10	3	—	—	—	—	+	−	—	—	—		—	—	—	—	—	4.7
		24	4	0.3	0.3	0.7	—	+	〃	1.0396	—	—		—	—	—	—	—	14.7
		28	5	0.7	0.2	0.5	—	+	〃	1.0356	—	—		—	—	—	—	—	6.7
		34	6	—	—	—	—	+	〃	1.036	—	—		—	—	—	—	—	5.2
		48	7	—	—	—	—	+	〃	1.0355	—	—		—	—	—	—	—	18.8
		52	8	—	—	—	—	+	〃	1.0364	—	—		—	—	—	—	—	7.1
		58	9	—	—	—	—	−	〃	1.0347	—	—		—	—	—	—	—	5.8
		72	10	—	—	—	—	〃	〃	1.0334	—	—		—	—	—	—	—	21.0
		76	11	—	—	—	6.18	〃	〃	1.0347	—	—		—	—	—	—	—	7.6
		82	12	—	—	—	—	〃	〃	1.0324	—	—		—	—	—	—	—	5.5
VI	1	Immediately after	1	0.8	1.0	0	—	+	+	1.071	27.47	18.66	6.69	5.50	6.47	5.97	1.83	1.01	0.2
		17	2	0.6	0.8	0	7.43	+	−	1.045	11.83	4.50	3.16	0.30	1.04	3.87	2.64	0.82	1.1
		21	3	0.3	0.3	0.6	6.26	+	〃	1.0395	12.49	4.26	3.68	0.08	0.50	3.60	2.84	0.79	5.3
		34	4	0.4	0.3	0.1	6.14	+	〃	1.038	12.06	—	4.16	—	0.51	3.61	3.05	0.73	5.5
		39	5	0.7	0.4	0.9	6.38	+	〃	1.036	12.43	3.17	2.62	0.03	0.32	4.31	4.17	0.78	3.3
		44	6	0.3	0.1	0.3	6.09	+	〃	1.038	12.72	—	3.04	—	0.57	5.04	3.32	0.75	8.4
		57	7	0.1	0.1	0.6	7.89	+	〃	1.037	—	—	—	—	—	—	—	—	4.0
		62	8	0.3	0.1	0.4	7.94	+	〃	1.025	—	—	—	—	—	—	—	—	2.0

VI	1	67	9	0.1	0	1.0	8.01	+	"	1.035	—	—	—	—	—	—	—	—	9.8
		80	10	—	—	—	8.06	+	"	1.037	—	—	—	—	—	—	—	—	5.7
		85	11	0.1	0.2	2.0	8.04	−	"	1.035	—	—	—	—	—	—	—	—	3.5
		90	12	0.1	0.2	2.0	7.90	"	"	1.032	—	—	—	—	—	—	—	—	10.0
		103	13	0.2	—	0.9	8.02	"	"	1.035	—	—	—	—	—	—	—	—	5.2
		108	14	0.1	0.1	0.9	8.09	"	"	1.030	—	—	—	—	—	—	—	—	4.3
VII	5	Immediately after	1	2.0	2.0	0	6.17	+	+	1.0692	22.40	15.23	—	—	—	1.04	2.00	1.13	4.5
		24	6	1.8	1.8	0	6.15	+	+	1.0630	22.75	12.83	—	—	—	6.88	2.35	0.69	1.5
		28	10	1.4	1.4	0	6.17	+	−	1.0424	16.20	6.99	—	—	—	5.04	3.35	0.82	2.6
		72	14	1.1	1.1	0	6.20	−	"	1.0310	19.28	7.27	—	—	—	8.07	3.09	0.85	3.7
		96	18	0.8	0.8	0	6.22	"	"	1.035	15.50	5.79	—	—	—	5.76	3.14	0.81	3.7
		120	22	0.8	0.6	0.6	6.38	"	"	1.0347	14.59	6.62	—	—	—	3.78	3.48	0.71	4.0
		144	26	0.8	0.5	0.5	6.38	"	"	1.0322	18.36	6.94	—	—	—	7.38	3.24	0.80	4.8
VIII	8	Immediately after	1	—	—	—	—	+	+	1.071	25.23	16.81	5.17	11.64	6.29	1.05	1.08	13.5	
		25	6	—	—	—	—	+	−	1.039	14.54	4.83	3.84	0.99	7.56	1.27	0.87	6.0	
		49	10	—	—	—	—	−	"	1.035	13.47	3.43	2.54	0.89	6.06	3.09	0.89	2.7	
		73	14	—	—	—	—	"	"	1.034	13.59	4.03	3.35	0.68	4.46	4.20	0.90	7.8	
		97	18	—	—	—	—	"	"	—	—	—	—	—	—	—	—	6.5	
		121	22	—	—	—	—	"	"	—	—	—	—	—	—	—	—	7.6	
IX	2	Immediately after	1	—	—	—	—	−	−	1.0562	27.40	19.11	—	—	3.88	—	0.97	12.2	
		15	3	—	—	—	—	"	"	1.036	16.14	4.87	3.14	1.73	4.79	5.75	0.72	3.0	
		39	7	—	—	—	—	"	"	1.028	15.06	3.75	2.14	0.61	4.63	5.90	0.78	5.0	
		63	11	—	—	—	—	"	"	1.034	14.63	3.13	2.74	0.39	5.51	5.12	0.87	4.4	
		87	15	—	—	—	—	"	"	1.034	15.31	4.11	3.44	0.67	4.55	5.84	0.81	5.5	
		111	19	—	—	—	—	"	"	1.034	14.09	3.67	—	—	4.42	5.30	0.70	6.0	
		135	23	—	—	—	—	"	"	1.035	14.34	4.41	3.80	0.61	4.03	5.17	0.73	6.5	

TABLE 2—*Continued*

NUMBER OF EXPERIMENT	NUMBER OF PARTURITION	HOURS AFTER PARTURITION	NUMBER OF MILKING	COLOR			pH	ALCOHOL TEST	BOILING TEST	SPECIFIC GRAVITY	TOTAL SOLID	TOTAL PROTEIN	CASEIN	LACTO-GLOBULIN	ALBUMIN	FAT	LACTOSE	ASH	MILK QUANTITY
				Red	Yellow	Blue					per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	pounds
X	6	Immediately after	1	1.2	1.2	0	6.61	+	+	1.0695	23.12	19.46	7.40		12.06	3.52	1.28	0.86	16.2
		15	3	1.0	1.2	0	6.00	+	+	1.0482	21.23	11.68	5.79		5.89	5.98	2.75	0.82	1.0
		39	7	0.6	0.5	0.6	6.17	+	−	1.0340	13.67	4.06	3.28		0.78	—	—	0.73	9.0
		63	11	0.6	0.4	0.7	6.26	−	"	1.0317	15.07	3.91	1.99		1.92	5.31	5.11	0.74	12.0
		87	15	0.5	0.4	0.7	5.74	"	"	1.0334	15.35	3.86	3.00		0.86	6.46	4.28	0.75	15.0
		111	19	—	—	—	—	"	"	1.0350	15.05	4.32	3.64		0.68	5.84	4.14	0.75	20.0
XI	1	Immediately after	1	—	—	—	—	+	+	1.044	21.62	11.54	5.86	5.68		6.92	2.23	0.93	2.0
		28	6	—	—	—	—	+	−	1.043	16.68	6.85	3.02	3.83		5.06	3.83	0.94	6.6
		52	10	—	—	—	—	−	"	1.034	13.13	3.17	—	—		—	—	0.72	9.3
		76	14	—	—	—	—	"	"	1.034	14.57	5.05	3.92	0.12		5.55	4.22	0.75	10.3
		100	18	—	—	—	—	"	"	1.033	13.70	3.75	2.87	0.88		—	—	0.81	9.0
		124	22	—	—	—	—	"	"	1.036	14.01	3.76	3.17	0.58		—	—	0.73	9.6
		148	26	—	—	—	—	"	"	1.034	15.25	3.81	3.20	0.62		6.01	4.70	0.73	14.0
		172	29	—	—	—	—	"	"	1.032	15.70	3.78	3.19	0.59		—	—	0.65	13.7
		196	32	—	—	—	—	"	"	1.037	14.61	4.00	3.46	0.54		—	—	0.75	16.6
		220	36	—	—	—	—	"	"	1.033	14.80	3.77	3.03	0.74		6.39	3.93	0.71	13.4
XII	8	Immediately after	1	—	—	—	—	+	+	1.065	21.89	15.73	6.17		10.56	3.92	1.26	0.98	5.7
		26	6	—	—	—	—	+	−	1.055	19.85	8.68	—		—	—	—	1.01	5.7
		50	10	—	—	—	—	+	"	1.040	15.02	4.89	3.85		1.04	5.37	3.78	0.98	8.5
		74	14	—	—	—	—	−	"	1.035	14.30	4.13	3.33		0.80	5.73	3.63	0.81	7.5
		98	18	—	—	—	—	"	"	1.031	14.36	3.64	3.13		0.51	5.11	4.82	0.79	8.5
		112	22	—	—	—	—	"	"	1.033	13.55	4.52	—		—	—	—	0.76	10.1
		136	26	—	—	—	—	"	"	1.034	14.24	3.48	3.00		0.48	6.11	3.90	0.75	11.0

XIII	1	Immediately after	1	—	—	—	—	+	+	1.0567	21.28	14.18	6.54	7.64	3.50	2.56	1.04	12.4
		15	3	—	—	—	—	+	−	1.036	15.48	6.34	4.98	1.36	4.44	3.80	0.90	3.7
		39	7	—	—	—	—	+	〃	1.032	13.82	4.83	3.65	1.18	—	—	0.81	8.0
		63	11	—	—	—	—	−	〃	1.035	13.08	4.69	3.85	0.84	4.81	2.70	0.88	6.5
		87	15	—	—	—	—	〃	〃	1.034	13.50	4.38	3.95	0.43	5.84	2.54	0.74	8.5
		111	19	—	—	—	—	〃	〃	1.035	13.62	3.90	3.40	0.50	5.79	3.14	0.76	12.0
		135	23	—	—	—	—	〃	〃	1.0325	12.80	3.48	3.97	0.51	4.59	4.03	0.70	11.0
		159	27	—	—	—	—	〃	〃	1.032	13.54	3.41	2.84	0.57	4.62	4.79	0.72	8.7
XIV	6	Immediately after	1	—	—	—	—	+	−	1.0375	17.36	10.84	4.57	6.27	3.58	1.94	1.00	3.2
		17	3	—	—	—	—	+	〃	1.033	14.74	5.99	5.47	0.52	5.13	2.76	0.86	6.3
		41	7	—	—	—	—	+	〃	1.034	15.30	4.75	4.45	0.30	5.87	3.84	0.84	10.5
		65	11	—	—	—	—	−	〃	1.033	16.00	3.50	3.24	0.26	—	—	0.81	10.0
		89	15	—	—	—	—	〃	〃	1.033	15.20	3.95	3.49	0.46	—	—	0.68	13.0
		113	19	—	—	—	—	〃	〃	1.032	14.56	3.30	2.99	0.31	—	—	0.72	10.7
		137	23	—	—	—	—	〃	〃	1.032	14.34	3.38	2.61	0.77	—	—	0.78	7.4
XV	9	Immediately after	1	—	—	—	—	+	+	1.0567	22.20	14.65	—	—	—	—	0.91	4.5
		27	5	—	—	—	—	+	−	1.0358	17.98	7.48	4.24	3.24	—	—	0.81	1.5
		51	9	—	—	—	—	+	〃	1.0320	16.70	4.77	3.92	0.85	—	—	0.74	2.6
		75	13	—	—	—	—	−	〃	1.034	13.42	4.16	3.64	0.52	—	—	0.70	3.7
		99	17	—	—	—	—	〃	〃	1.032	12.82	4.25	3.50	0.75	—	—	0.72	3.7
		128	21	—	—	—	—	〃	〃	1.033	13.36	3.51	3.07	0.44	—	—	0.78	4.0
		147	26	—	—	—	—	〃	〃	1.031	13.78	3.33	3.01	0.31	—	—	0.71	4.8
XVI	1	Immediately after	1	0.3	0.3	0.3	6.14	+	+	1.059	18.92	11.73	5.11	6.62	3.71	2.50	0.98	14.0
		16	6	0.3	0.3	0.3	6.24	+	−	1.0485	16.94	5.63	4.69	0.94	5.89	4.56	0.86	10.5
		40	10	0.3	0.3	0.3	6.33	+	〃	1.038	14.70	4.35	3.93	0.42	6.67	2.80	0.88	10.5
		64	14	0.3	0.1	0.5	6.34	−	〃	1.0345	13.90	4.03	3.30	0.73	—	—	0.80	11.6
		88	18	0.3	0.1	0.5	6.32	〃	〃	1.0325	14.06	3.29	3.05	0.24	5.36	4.61	0.80	12.0
		112	22	0.4	0.1	0.6	6.35	〃	〃	1.034	13.64	3.55	2.56	0.79	6.12	3.18	0.79	13.8
		136	26	0.3	0.1	0.6	6.35	〃	〃	1.0325	13.84	3.44	2.68	0.76	4.44	5.20	0.76	15.6

TABLE 2—*Concluded*

NUMBER OF EXPERIMENT	NUMBER OF PARTURITION	HOURS AFTER PARTURITION	NUMBER OF MILKING	COLOR			pH	ALCOHOL TEST	BOILING TEST	SPECIFIC GRAVITY	TOTAL SOLID	TOTAL PROTEIN	CASEIN	LACTO-GLOBULIN	ALBUMIN	FAT	LACTOSE	ASH	MILK QUANTITY
				Red	Yellow	Blue					*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*pounds*
XVII	6	Immediately after	1	—	—	—	—	+	+	1.059	21.56	12.06	—		—	6.69	1.92	0.89	4.0
		26	6	—	—	—	—	+	−	1.038	15.04	5.33	—		—	4.52	4.37	0.82	1.0
		50	10	—	—	—	—	+	"	1.037	14.00	3.96	—		—	4.46	4.73	0.85	1.0
		74	14	—	—	—	—	+	"	1.036	14.40	4.48	—		—	4.50	4.57	0.85	1.5
		98	18	—	—	—	—	−	"	1.036	—	—	—		—	—	—	—	2.3
		122	22	—	—	—	—	"	"	—	—	—	—		—	—	—	—	2.4
XVIII	9	Immediately after	1	0.7	0.4	0	5.01	+	−	—	—	—	—		—	—	—	—	10.5
		32	6	1.3	0.2	0	6.05	+	"	1.034	13.65	3.94	—		—	4.89	3.91	0.92	4.0
		56	10	1.0	0.7	0	6.58	−	"	1.0324	13.25	4.10	—		—	4.66	3.85	0.63	6.2
		80	14	0.8	0.4	0.5	6.56	"	"	1.0328	13.96	4.68	—		—	4.73	3.95	0.63	5.5
		104	18	—	—	—	—	"	"	1.033	—	—	—		—	—	—	—	6.2
		128	22	—	—	—	—	"	"	1.032	—	—	—		—	—	—	—	6.2

TABLE 3

The presence of certain agglutinins in normal bovine colostrum

NUMBER OF EXPERIMENT	COW		SAMPLE		ORGANISMS USED AS ANTIGENS IN TESTING FOR AGGLUTININS IN COLOSTRUM				
	Breed	Number of parturition	Hours after parturition	Number of milking	*Bact. coli*	*Bact. paratyphosum* B	*Bacillus abortus*	*Bacillus melitensis*	*Vibrio fetus*
1	Ayrshire 94	4	0	1	640	80	20	40	
			4	2	640	160	20	—	
			12	3	160	80	10	—	
			24	4	80	40	—	—	
			29	5	40	40			
			34	6	20	10			
			48	7	20	20			
			53	8	10	—			
			58	9	—	—			
			72	10	—	—			
			77	11	—	10			
			82	12	—	—			
			96	13	—	—			
				14		—			
2	Ayrshire 94	5	19*		160	40	10	40	320
			0	1	320	40	80	40	1,280
			9	2	80	40	20	40	80
			33	3	10	10	10	—	20
			48	4	—	—	—	10	20
			51	5	—			—	—
			57	6	—				—
3	Ayrshire 86	6	18	3	160	40	20	20	320
			33	4	80	40	—	—	640
			37	5	40	20	—	—	80
			57	6	10	—			40
			61	7	—				20
			66	8					10
			81	9					10
			85	10					10
			90	11					—
4	Ayrshire 115	1	12	3	640	80	80	320	1,280
			39	4	80	20	20	40	80
			43	5	20	—	—	—	160
			63	6	10	—	—	—	80
			67	7	—				40
			72	8					20
			76	9					10
			96	10					—
			106	11					—

* This milk was drawn 19 hours before parturition.

TABLE 3—*Continued*

NUMBER OF EXPERIMENT	COW		SAMPLE		ORGANISMS USED AS ANTIGENS IN TESTING FOR AGGLUTININS IN COLOSTRUM				
	Breed	Number of parturition	Hours after parturition	Number of milking	*Bact. coli*	*Bact. paratyphosum* B	*Bacillus abortus*	*Bacillus melitensis*	*Vibrio fetus*
			0	1	160	40	160	80	640
			4	2	80	40	80	80	160
			10	3	40	10	10	10	20
			24	4	20	10	10	10	40
5	Ayrshire 110	2	28	5	—	—	—	—	20
			34	6	—				10
			48	7					10
			52	8					—
			0	1	640	160	40	80	2,560
			17	2	160	80	10	20	320
			21	3	40	20	10	20	160
			34	4	20	—	—	—	40
6	Ayrshire 116	1	39	5	10	—			20
			44	6	10	—			20
			57	7	—	—			10
			62	8					10
			67	9					—
			0	1	20	10	—	—	
			24	6	40	—	10	—	
7	Guernsey 173	5	48	10	—	—	—		
			72	14	—				
			0	1	640	80	20	40	160
			25	6	10	—	—	10	10
8	Guernsey 168	8	49	10	—			10	10
			73	14	10			—	10
			97	18	10				10
			15	3	10	—	—	—	20
			39	7	—	—	—	—	20
9	Guernsey 189	2	63	11	—				10
			87	15	—				10
			111	19					—
			0	1	40	10	40		—
			15	3	10	—	10		—
10	Holstein 195	6	39	7	—		—		
			63	11					
			87	15					

TABLE 3—*Concluded*

NUMBER OF EXPERIMENT	COW		SAMPLE		ORGANISMS USED AS ANTIGENS IN TESTING FOR AGGLUTININS IN COLOSTRUM				
	Breed	Number of parturition	Hours after parturition	Number of milking	*Bact. coli*	*Bact. paratyphosum B*	*Bacillus abortus*	*Bacillus melitensis*	*Vibrio fetus*
11	Holstein 237	1	0	1	160	—	20		—
			28	6	80	—	10		—
			52	10	20		—		
			76	14	10				
			100	18	—				
12	Holstein 171	8	0	1	160	20	40	40	640
			26	6	160	10	40	20	160
			50	10	—	—	10	10	10
			74	14	—		—	—	10
			98	18				—	—
			112	22					—
13	Holstein 243	1	0	1	1,280	20	20	40	640
			15	3	40	10	—	—	40
			39	7	—	—		—	—
			63	11		—			20
			87	15					—
14	Holstein 197	6	0	1	10	—	—	—	—
			17	3	—	—	—	—	—
15	Holstein 173	9	0	1	160	80	20	20	320
			27	5	40	20	10	10	80
			51	9	10	—	—	—	10
			75	13	10	—			10
			99	17	—				—
			128	21	—				—
16	Holstein 239	1	0	1	160	20	20	20	320
			16	6	10	—	—	—	40
			40	10	—				—
			64	18					—
									—
17	Guernsey 170	6	0	1	40	20	—	10	
			26	6	80	10	—	20	
			50	10	80	20	10	40	
			74	14	80	—	10	20	
18	Holstein 169	9	0	1	—	—	—	—	
			32	6	—	—	—	—	
Control	Rennet				—	—	—	—	—

immunized with horse erythrocytes, the serum being made inactive as the complement, fresh serum (0.05 cc.) of guinea-pig was used. The hemolysin-titre of the rabbit serum and its complement demonstrating power are indicated in the following table:

Hemolysin-titre

	DILUTION OF SERUM								
	50	100	200	400	800	1600	3200	6400	12800
Result of hemolysis.	+++	+++	+++	+++	+++	++	+	±	−

The complement demonstrating power

A survey was made as to the existence or non-existence of hemolysis by mixing suitable quantity of fresh serum of cow.

	AMOUNT OF COMPLEMENT IN COW					
	0.05 cc.	0.01 cc.	0.005 cc.	0.001 cc.	0.0005 cc.	0.0001 cc.
Result of hemolysis.....	+++	+++	+++	+	−	−

Two-tenths cubic centimeter of amboceptor diluted 40 times and 1 cc. of 1 per cent suspension of horse erythrocytes (total quantity 2.2 cc.) were used for the purpose.

In order to determine the presence of complement in colostrum, different quantities of colostrum (1.0, 0.8, 0.6, 0.4 and 0.2 cc., respectively) were put separately into fine test tubes. Proper quantities of physiological salt solution were then added to the colostrum contained in each tube, the quantity of the contents of each tube being made uniform at 1 cc. and again to the contents of each tube was added 0.2 cc. each of immune serum or amboceptor diluted 40 times (dilution was resorted to each time immune serum was used for similar purposes). Adding to the mixture 1 cc. suspension of horse erythrocytes and completely mixing the entire contents, the mixture was incubated at 37°C. for 1 hour, and after keeping in an ice-room for 15 hours the contents of each tube was examined. Throughout the examination the centrifugal separation process was carried out, constant care being taken to assure the precipitation of erythrocytes to the bottom of the

tubes. For the purpose of contrast fresh serum of a guinea-pig and a mixture of physiological salt solution (1 cc.) and suspension of erythrocytes (1 cc.) were provided as the substitutes for the colostrum in order to prove the normality of immune serum and erythrocytes. (With the former the result of hemolysis was satisfactory, the case being the reverse with the latter.)

The above experiment was applied to all of the colostrum put to test, the result, however, proving negative throughout, whereas with the addition of fresh serum (0.05 cc.) extracted from a guinea-pig to the contents of each tube the result of hemolysis was complete.

RESULTS

The results are summarized hereunder:

1. In the normal parturition, the colostrum always contains agglutinins.

2. The period of the presence of agglutinins in the colostra ranges from 1 to 4 days after parturition.

3. Agglutinins are contained in the colostra principally after parturition; but, as a rule, they speedily decrease in number afterward and finally disappear.

4. Generally, agglutinins reacting with *Bacterium coli* and *Vibrio fetus* are found to be most abundant, those reacting with *Bact. paratyphosum B*, *Bacillus melitensis* and *Bacillus abortus* coming next in the named order.

5. The breeds of cattle and the number of parturition apparently have nothing to do with the quantity of agglutinins and the length of the period of their existence in the colostra after parturition.

6. In the colostrum obtained from a cow suffering from the inflammation of the udder there seems to be no fixed rule as to the appearance of agglutinins.

7. In the milk secreted by a cow which aborted in the fourth month after pregnancy, the presence of agglutinins has not been proved.

8. A large quantity of agglutinins was found in the milk secreted 19 hours before parturition.

9. Throughout the series of experiments made by the authors with fresh and normal colostra the presence of complements were not proved.

10. Colostra of the same and of different cows are exceedingly variable in chemical composition. The specific gravity and total ash are fairly constant. There were wide fluctuations in the hydrogen ion concentration, color, coagulation, the percentage of total proteins and the relative proportions of each, the percentage of lactose, and the fat content.

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CALCULATING RATIONS FOR DAIRY COWS*

T. E. WOODWARD

Bureau of Dairy Industry, United States Department of Agriculture

In many dairy-cow investigations, the animals must be fed according to some definite plan, if accurate comparisons are to be made of the production of dams and daughters and the effect of various feeds over entire lactation periods. The usual method of feeding is to select some standard, and then to provide nutrients in such amounts as will meet the requirements of this standard. In some experiments, a little more than the required amount is fed to make sure that the animal receives sufficient nutrients for maintenance and milk production.

Because of the amount of work involved in calculating nutrient requirements for large numbers of animals, a quick and easy method of figuring rations was needed at the Bureau of Dairy Industry Experiment Farm at Beltsville, Maryland. After some study and the trying out of different methods, a system was developed (table 1) by which the rations could be easily figured at the rate of one a minute, if the weight of the cow and the quantity and quality of milk produced were known. These rations, when compared with those obtained by exact calculations, are ordinarily accurate to within one pound of grain per cow per day. This method has been used successfully at Beltsville for several years. It is now passed along with the thought that it may be of some value to other investigators. Although the system given has been prepared to fulfill the requirements of the Savage standard as to total digestible nutrients, there is no reason why a similar method can not be used for any standard desired.

The feeds generally used at Beltsville are No. 2 alfalfa hay, good corn silage, dried beet pulp, and two grain mixtures. The total digestible nutrients of the feeds, according to Henry and Morrison's "Feeds and Feeding," are as follows: beet pulp, 71.6 per

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TABLE 1

Number of pounds alfalfa hay required daily (Savage Standard) for cows producing different quantities of milk varying in butterfat test

MILK	BUTTERFAT TEST—PER CENT																			
	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8	6.0	6.2	6.4	6.6	6.8	7.0
lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
10	6	6	6	6	7	7	7	7	7	8	8	8	8	8	9	9	9	9	9	10	10
11	6	6	7	7	7	7	8	8	8	8	9	9	9	9	10	10	10	10	10	11	11
12	7	7	7	8	8	8	8	9	9	9	9	10	10	10	10	11	11	11	11	12	12
13	7	7	8	8	8	8	9	9	9	10	10	10	11	11	11	12	12	12	12	13	13
14	8	8	8	9	9	9	10	10	10	11	11	11	12	12	12	12	13	13	13	14	14
15	8	9	9	9	10	10	10	11	11	11	12	12	12	13	13	13	14	14	14	15	15
16	9	9	10	10	10	11	11	11	12	12	12	13	13	13	14	14	14	15	15	15	16
17	9	10	10	10	11	11	12	12	12	13	13	13	14	14	15	15	15	16	16	16	17
18	10	10	11	11	12	12	13	13	13	14	14	14	15	15	16	16	16	17	17	17	18
19	11	11	11	12	12	13	13	14	14	14	15	15	15	16	16	17	17	18	18	18	19
20	11	12	12	13	13	14	14	15	15	15	16	16	16	17	17	18	18	19	19	19	20
21	12	12	13	13	14	14	15	15	15	16	16	17	17	18	18	19	19	19	20	20	21
22	12	13	13	14	14	15	15	16	16	17	17	18	18	19	19	19	20	20	21	21	22
23	13	13	14	15	15	16	16	17	17	18	18	18	19	19	20	20	21	21	22	22	23
24	13	14	14	15	16	16	17	17	18	18	19	19	20	20	21	21	22	22	23	23	24
25	14	14	15	16	16	17	18	18	19	19	20	20	21	21	22	22	23	23	24	24	25
26	14	15	16	16	17	17	18	19	19	20	20	21	21	22	23	23	24	24	25	25	26
27	15	16	16	17	18	18	19	20	20	21	21	22	22	23	23	24	24	25	26	26	27
28	16	16	17	18	18	19	19	20	20	21	22	22	23	23	24	25	25	26	26	27	28
29	16	17	17	18	19	20	20	21	21	22	23	23	24	24	25	26	26	27	28	28	29
30	17	17	18	19	20	20	21	22	22	23	24	24	25	25	26	27	27	28	28	29	30
32	18	18	19	20	21	22	22	23	24	24	25	26	26	27	28	28	29	30	30	31	32
34	19	20	21	21	22	23	24	25	25	26	27	27	28	29	29	30	31	31	32	33	33
36	20	21	22	23	24	24	25	26	27	28	28	29	30	30	31	32	33	33	34	35	35
38	21	22	23	24	25	26	27	28	28	29	30	31	31	32	33	34	34	35	36	37	37
40	22	23	24	25	26	27	28	29	30	31	31	32	33	34	35	35	36	37	38	39	39
42	23	24	25	26	27	28	29	30	31	32	33	33	34	35	36	37	38	39	40	41	41
44	24	25	27	28	29	30	31	32	33	34	35	35	36	37	38	39	40	41	42	43	43
46	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	44	45
48	27	28	29	30	31	33	34	35	36	37	38	39	40	41	41	43	44	44	45	46	47
50	28	29	30	31	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
52	29	30	31	33	34	35	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
54	30	31	33	34	35	37	38	39	40	41	42	43	44	46	47	48	49	50	51	52	
56	31	32	34	35	37	38	39	41	42	43	44	45	46	47	48	50	51	52	53		
58	32	34	35	36	38	39	41	42	43	44	46	47	48	49	50	51	53	54			
60	33	35	36	38	39	41	42	43	45	46	47	48	49	51	52	53	54				
64	36	37	39	40	42	43	45	46	48	49	50	51	53	54	55	57					
68	38	40	41	43	44	46	48	49	50	52	53	55	56	57	59						
72	40	42	43	45	47	49	51	52	53	55	57	58	59	61							
76	42	44	46	48	50	51	53	55	57	58	60	61	63								
80	44	46	48	50	52	54	56	58	60	61	63										
84	47	49	51	53	54	57	59	61	62	64											
88	49	51	53	55	56	60	62	64	65												
92	51	53	55	57	59	63	65	66													
96	54	56	58	60	62	66	67														
100	56	58	60	63	65	68															

TABLE 1—*Continued*

	Weight	*Hay*
For maintenance	700	11
	800	12
	900	14
	1,000	15
	1,100	17
	1,200	18
	1,300	20
	1,400	21
	1,500	23

cent; corn silage, 17.7; and alfalfa hay, 51.6. From Henry and Morrison, also, it is estimated that the grain mixtures contained 73.1 and 73.7 per cent digestible nutrients. In total digestible nutrients, 1 pound of alfalfa is equal to about 0.7 pound of grain or beet pulp, or to about 3 pounds of silage.

The following examples show how the information contained in table 1 is used:

Example 1. Daily ration for cow weighing 1,500 pounds and giving 50 pounds milk testing 3 per cent butterfat

Requirements in terms of hay:

Maintenance	23 pounds
Milk	28 pounds
Total	51 pounds

Roughage received:

Silage	45 pounds, equivalent to 15 pounds hay
Hay	18 pounds
Total	33 pounds

51 − 33 = 18 pounds hay, the equivalent of which is to be made up of grain 18 pounds × 0.7 = 12.6 pounds, the quantity of grain required. The ration, then, is 12.6 pounds of grain, 18 pounds of hay, and 45 pounds of silage. The exact requirements, according to the Savage standard, when the analyses stated in "Feeds and Feeding" are used, are 26.24 pounds digestible nutrients; the ration given contains 26.54 pounds.

Example 2. Daily ration for cow weighing 1,100 pounds and giving 20 pounds of milk testing 4 per cent butterfat

Requirements in terms of hay:

Maintenance	17 pounds
Milk	14 pounds
Total	31 pounds

Roughage received:

Silage	33 pounds, equivalent to	11 pounds hay
Hay		14 pounds
Total		25 pounds

31 − 25 = 6 pounds to be made up in grain.
6 × 0.7 = 4.2 pounds, the amount of grain given.
Nutrients required, 15.72 pounds; nutrients in ration given, 16.16 pounds.

Example 3. Daily ration for cow weighing 800 pounds and giving 30 pounds of milk testing 5 per cent butterfat

Requirements in terms of hay:

Maintenance	12 pounds
Milk	24 pounds
Total	36 pounds

Roughage received:

Silage	24 pounds, equivalent to	8 pounds hay
Hay		10 pounds
Total		18 pounds

36 − 18 = 18 pounds to be made up in grain.
18 × 0.7 = 12.6 pounds, the amount of grain needed to complete the ration.
If 4 pounds beet pulp are fed, this will leave 8.6 pounds grain. Nutrients required, 18.49 pounds; nutrients in ration given, 18.61 pounds.

In the three examples shown above, the nutrients given exceeded those required by 0.3 pound, on an average. This is no more than would be needed for the production of 1 pound of milk.

The method is thought to be accurate enough for investigations made with feed for which no special chemical analyses are given. No doubt the variation in composition of feeds results in a larger error than does the use of this method.

THE FEATHERING OF EVAPORATED MILK IN HOT COFFEE*

RANDALL WHITAKER

Research Laboratories, National Dairy Products Corporation Baltimore, Maryland

Milk, cream, and evaporated milk occasionally curdle or "feather" when placed in hot beverages such as coffee. The term "feather" is descriptive of the fluffy, feather-like appearance of the curd particles which form under certain conditions. The subject of the occasional feathering of evaporated milk in hot coffee has received practically no attention by scientific workers, as indicated by the absence of published experimental work. Hunziker (4) recognizes the feathering of evaporated milk but does not include it as a defect of this product. He states, while discussing the manufacture of this product, that feathering is usually associated with high viscosity and the use of excessive homogenization pressures. He also reports that the use of an homogenizer with a spring controlled valve induces feathering.

The feathering of cream in hot coffee has been studied by several investigators (1), (3), (7), (8). It has been shown that acidity of the cream, extent of homogenization, heat treatment, fat content, extent of fat globule clumping, salt balance, method of adding the cream to the coffee and the kind of water used in preparing the coffee influence the feathering of cream in coffee. Evaporated milk is used in much the same manner as cream. This product, although containing relatively more solids-not-fat and less fat, behaves in coffee quite similar to fresh cream in some respects.

In this paper is recorded the observations made during an investigation of the causes of and conditions necessary for the feathering of evaporated milk in hot coffee.

COAGULATION OF EVAPORATED MILK IN COFFEE

On account of their protein content, fluid dairy products are susceptible to coagulation by heat. The temperature at which

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coffee is served is sufficiently high to occasionally cause partial coagulation of the milk, cream, evaporated milk, etc. used in it. Under certain conditions, there results well defined curd particles which give rise to the phenomena known as feathering.

Because of the relatively greater concentration of the coagulable protein in evaporated milk, the feathering of this product is more noticeable and generally more pronounced than is the feathering of milk and cream. Heat coagulation of milk has been studied by Sommer and Hart (6). The salt balance, which may be briefly described as the relative amounts of available calcium and magnesium as compared with the phosphates and citrates, has been shown to be of paramount importance. These workers contend that when the calcium and magnesium is high, the milk can be stabilized by the addition of citrates and phosphates. In like manner adding soluble calcium and magnesium will stabilize milks which are high in citrates and phosphates. Doan (2) and Tracy and Ruehe (7) have supplied proof that the decreased stability resulting from homogenization is caused by an increase in calcium and magnesium resulting from the absorption of the phosphates, citrates, and casein on the increased surface presented by the homogenized fat. In like manner the decreased stability noted with high acidity, is attributable to the increased availability of the divalent cations in the acid reaction.

It is contended by some workers (5) that there are other factors besides the salt balance such as acidity, which are concerned with heat coagulation. It may be said, however, that in practically every case, it is possible to alter the heat stability of a milk by effecting a change in the salt balance.

Using the salt balance theory as a basis it may be predicted that any factor which tends to increase the amount of available calcium will result in increased feathering. This may be brought about by either directly increasing the available calcium or by removing such anions as phosphate, citrate, etc. which keep calcium in an unavailable form. The addition of such salts as sodium citrate and secondary sodium phosphate will reduce the available calcium and the tendency to feather.

THE FEATHERING TEST

Prior to making a study of the factors concerned with the feathering of evaporated milk in hot coffee, it was deemed advisable to have some means of measuring the susceptibility of a milk to feather in hot coffee. A feathering test was therefore devised. This test was based on the previously noted influence of the salt balance and was not dependent upon an acidity effect. If a milk failed to feather when placed in hot coffee, it was made to do so by the addition of certain salts; when feathering was observed, the addition of certain other salts would prevent it. By the addition of a known amount of the specific salt a measure of the susceptibility of the milk to feather was thus obtained.

The coffee used in this test was prepared by heating to the boiling point 250 cc. of distilled water and 10 grams of ground coffee and immediately straining through fine cheese cloth. The coffee thus prepared was kept at 95 to 100°C. until used.

When a sample of evaporated milk was to be tested, 5 cc. of milk was diluted with 1 cc. of distilled water. One cubic centimeter of this mixture was then rapidly added to 10 cc. of the hot coffee solution and immediately stirred. If the appearance of the coffee in the test tube was uniform throughout and there was no evidence of feathering, the test was repeated, diluting another 5 cc. portion of the milk with 1 cc. of a 1:9 solution of calcium chloride in place of the 1 cc. of distilled water. The 1:9 calcium chloride solution was prepared by mixing 1 cc. of N/2 $CaCl_2$ and 9 cc. of distilled water. If the coffee again failed to feather, the test was repeated using 1 cc. of a 2:8 $CaCl_2$ solution. This procedure was continued until feathering was noted. The number of cc. of N/2 $CaCl_2$ used in making the 10 cc. stock solution was taken as the unit of the test. The result of the test was then expressed as the number of these units which first caused feathering.

If the milk was found to feather without the addition of $CaCl_2$ then N/2 sodium citrate was used. Increasing amounts were added in the manner described above until feathering failed to appear.

To distinguish between the calcium chloride units and the sodium citrate units, a minus sign (−) was prefixed to the calcium chloride values, denoting that the milk did not feather normally, while a positive sign (+) was prefixed to the citrate values to indicate a positive feathering test. Table 1 illustrates several selected examples. This test is a measure of the susceptibility of a given sample of evaporated milk to feather. As indicated in table 1, milks having minus values do not feather, while positive values show this defect. The extent to which a given sample does or does not feather is indicated by the magnitude of the number given. For uniform and reproducible results the procedure as outlined must be adhered to rigidly.

TABLE 1

Illustration of feathering test values

FEATHERING VALUES		STOCK SOLUTION: N/2 salt solution (parts)	STOCK SOLUTION: Distilled water (parts)	
−3	Feathering produced by 1 cc. of	3	7	Calcium chloride
−2		2	8	
−1		1	9	
+1	Feathering produced by 1 cc. of	1	9	Sodium citrate
+2		2	8	
+3		3	7	

FACTORS WHICH INFLUENCE FEATHERING

Experimental work revealed several factors which influenced the extent to which evaporated milk feathered when placed in hot coffee. These factors, with a brief discussion, are as follows:

1. *Salt balance of the milk.* As previously pointed out a milk which has an excess of available calcium and magnesium will tend to feather when placed in hot coffee. An excess of calcium may be due to the natural salt content of the milk, to the addition of a calcium neutralizer before condensing, to high acidity, to depletion of opposing anions on homogenization, etc. The effect of

excess calcium was demonstrated in this work by the fact that with every sample of milk examined, the addition of a soluble calcium salt, such as the chloride, produced increased feathering whereas the tendency to feather was reduced when the available calcium was removed from the sphere of action by the addition of such substances as secondary sodium phosphate, sodium citrate, sodium hydroxide, etc. Evaporated milk which was unstable due to excessive amounts of citrates and phosphates was not encountered. This form of unstability is very infrequent.

2. *Hardness of the water.* Tracy and Ruehe (7) found that the hardness of the water used in preparing coffee had an influence on

TABLE 2

The influence of the hardness of the water used in the preparation of the coffee on the feathering of evaporated milk

SOURCE OF WATER	HYDROGEN ION CONCENTRATION	HARDNESS OF WATER	FEATHERING TEST (1 CC. IN 10 CC.)
	pH	*p.p.m.**	
Distilled	6.9	0	−4
Baltimore, Md.	6.8	35	−3
Madison, Wis.	7.6	35	−4
Chicago, Ill.	7.3	80	−4
Detroit, Mich.	6.8	60	−4
New York City	6.5	20	−3
Tempe, Arizona	7.3	150	−3
Youngstown, Ohio	7.4	150	−4

* Calculated as calcium carbonate.

the feathering of cream. It has been shown that calcium (and magnesium) in solution tends to induce feathering in evaporated milk. In table 2 is shown the results of a feathering test performed on coffees made from samples of water, procured from six different cities. A single source of evaporated milk was used in this work. The hardness of each sample of water was estimated by means of its soap consuming power. It will be seen from this table that there was insufficient calcium in the waters collected to produce a change in the feathering value of the sample of milk used in this test.

3. *Concentration of the coffee.* Several people were requested

to indicate the quantity of ground coffee they recommended for making a given volume of coffee. The average ratio was 10 grams of ground coffee to 250 cc. of water. As little as 7 grams was recommended in one case, while one housewife used 13.5 grams for this amount of water. Investigation showed that evaporated milk tended to feather more readily in strong coffee than in weak. It seems probable that the salts and the tannic acid extracted from the coffee assist in the feathering of the evaporated milk.

4. *Method of preparing the coffee.* It has been suggested that substances extracted from coffee influence the extent of feathering. It was to be expected, therefore, that the method of prepar-

TABLE 3

Ash content and hydrogen ion concentration of coffee prepared by different methods (10 grams ground coffee and 250 cc. distilled water)

METHOD OF PREPARATION	TREATMENT OF COFFEE	ASH	HYDROGEN ION CONCENTRATION
		per cent	*pH*
Pot	Filtered immediately on boiling	0.13	4.93
	Filtered after standing 5 minutes	0.16	4.92
	Ditto, after 10 minutes	0.18	4.93
Percolated	Five minutes	0.14	4.92
	Ten minutes	0.14	4.95

ing the coffee would also have an effect, but only in so far as the salts and tannic acid were extracted from the coffee grounds. Considering the amount of water-soluble ash as a measure of extraction, it will be seen from table 3 that coffee prepared by the drip or percolation method was less liable to cause feathering than was coffee made by boiling in the pot. The data show that prolonged contact of the coffee and the grounds increased the quantity of soluble ash and consequently tended to increase feathering. The hydrogen ion concentration of the coffee remained constant and was independent of the method of preparation.

5. *Amount of evaporated milk used in coffee.* Several individuals

were requested to indicate the quantity of evaporated milk preferred in a cup of coffee of standard size. In table 4 will be found the quantities indicated when the milk was placed in the cup prior to adding the coffee, and when the quantity of milk selected was dependent upon the color produced in the coffee by the milk. The inconsistencies noted made the average quantity of evaporated milk used in coffee somewhat uncertain. For all practical purposes, however, it was assumed that one tenth as much milk as coffee was the average amount used.

TABLE 4

Quantity of evaporated milk used in a cup of coffee of standard size (150 cc.) by 11 individuals (10 grams ground coffee and 250 cc. water)

INDIVIDUAL	MILK ADDED PRIOR TO COFFEE	MILK ADDED TO COFFEE
	cc.	*cc.*
A	13	10
B	16	7
C	8	17
D	11	5
EQ	6	9
F	20	
G	19	
H	7	5
I	20	15
J		13
K		10
Average	13.3	10.1

Laboratory experiments indicated that feathering was always more pronounced when the smaller quantities of milk were used in the coffee. Better feathering conditions existed when a small amount of evaporated milk was added, because (*a*) under such conditions a relatively small amount of coagulable protein came in contact with a large quantity of acid, salt-containing coffee solution, (*b*) the resulting white curd particles were more noticeable against the darker background of the coffee and (*c*) the small quantity of evaporated milk was heated to a higher temperature than a larger volume would have been. From table 5 it will be seen that the pH of coffee-evaporated milk mixtures varies with

the amount of milk used in a given quantity of coffee. When small portions of milk are used, the casein of the evaporated milk is brought near the isoelectric point and in consequence, coagulation takes place readily. It is probable, therefore, that individuals using small quantities of evaporated milk in coffee will encounter feathering more frequently than those who use large quantities of milk.

6. *Method of mixing milk and coffee.* Laboratory tests showed that feathering was more frequent when the evaporated milk was

TABLE 5

Hydrogen ion concentration of various coffee-evaporated milk mixtures

COMPOSITION OF COFFEE-MILK MIXTURE				HYDROGEN ION CONCENTRATION
Amount of coffee	Amount of milk	One cc. of citrate solution	One cc. of $CaCl_2$ solution	
cc.	cc.			*pH*
10	0.0			4.9
10	0.1			5.1
10	0.5			5.4
10	0.7			5.6
10	1.0			5.7
10	2.0			5.8
10	1.0	1:9		5.7
10	1.0	2:8		5.7
10	1.0	3:7		5.7
10	1.0		1:9	5.7
10	1.0		2:8	5.7
10	1.0		3:7	5.6

added slowly, drop by drop, to the hot coffee. Adding the milk in such a manner without stirring created conditions which foster coagulation for the reasons given under (5). In some cases, agitation destroyed the curdled appearance, in others the effect was permanent. When feathering presisted on agitation the curd particles often lost their fluffiness and became grainy in appearance. The tendency to feather was less if the coffee was added to the milk than if the milk was poured into the hot coffee. The use of sugar in quantities normally used had no effect on the feathering of evaporated milk in coffee.

SUSCEPTIBILITY OF COMMERCIAL SAMPLES TO FEATHERING

Fifty-two cans of evaporated milk were tested for feathering in an attempt to estimate the extent to which the present day commercial product feathers in hot coffee. These cans, representing 25 different brands of evaporated milk, were purchased in stores in 7 cities throughout the United States. From table 6 it will be seen that only one sample was found to feather when 1 cc. of treated milk was tested in 10 cc. of coffee. The average feathering test value of all samples was −2.6. Some coffee drinkers prefer a smaller quantity of evaporated milk than was represented in these tests, and since feathering is more pronounced in such cases, some of the samples were tested by using 0.5 cc. of milk. When this small quantity of milk was used, milk feathering was encountered in about two thirds of the samples. However, the number of people using such a small quantity of milk is comparatively small. It seems probable that the average person using evaporated milk as now offered to the trade will encounter feathering but rarely when used in coffee which has been prepared in such a manner that it contains a minimum quantity of extracts such as tannic acid, salts, and the like.

In addition to the feathering test, other determinations were made on many of the samples. These determinations and the values obtained are included in table 6. An examination of the data in this table show that there is no apparent relationship between feathering as measured by the feathering test and any of the other physical constants that were determined.

From table 6, it will be seen that sample No. 1 was found to feather excessively. This sample was unusual in several respects. The milk was lumpy and possessed a coagulated appearance. It seemed probable that this sample was manufactured from milk of inferior quality, judging from the relatively high amino and ammonical nitrogen. Since the pH and the titratable acidity was normal, neutralization prior to condensing was indicated. The neutralization, probably accomplished with calcium and magnesium, decreased the heat stability of the milk and caused it to coagulate in the sterilizer. The excessive calcium-magnesium was likewise probably responsible for the marked feathering.

TABLE 6

Analysis of fifty-three samples of commercial evaporated milk

SAMPLE NUMBER	TOTAL SOLIDS	BUTTER FAT	ACIDITY	H ION CONCENTRATION	SPECIFIC GRAVITY	VISCOSITY	TOTAL N	AMMONIA N	AMINO N	FEATHERING TEST		PLATE COUNT	GENERAL REMARKS
										0.5 cc.	1.0 cc.		
	per cent	*per cent*	*per cent*	*pH*		*centipoises*	*per cent*	*per cent*	*per cent*			*cc.*	
1	27.4	8.4	0.37	6.00	1.056	43.0	0.99	0.010	0.048		+3	None	Coagulated, lumpy, flavor and odor normal
2	26.8	7.7	0.37	6.05	1.063	8.1	1.08	0.008	0.046		−1	Molds	Milk tasted thin and watery
3	28.5	7.9	0.35	6.03	1.062	8.3	1.08	0.007	0.048		−2	None	Fair quality
4	27.1	8.2	0.37	6.01	1.063	12.9	1.03	0.006	0.048		−2	None	Slight scorched flavor, color dark
4a										+2	−2		Good quality
4b										+1	−3		Good quality, crystals at bottom
4c										+1	−2		Good quality
5	25.4	7.3	0.31	6.04	1.056	6.1	1.00	0.006	0.037		−3	160	Rich flavor, fat churned slightly
6	28.4	8.1	0.35	6.08	1.069	11.2	1.05	0.005	0.046		−4	None	Fair flavor, fat churned
6a										−1	−4		Good quality
6b										−1	−3		Slight fat separation
6c										+1	−1		Fat churned and crystals at bottom
7	26.3	7.8	0.43	5.95	1.061	13.0	1.12	0.010	0.046		−4	None	Poor flavor, color dark, crystals
8	27.8	8.0	0.36	5.98	1.064	18.4	1.03	0.007	0.050		−3	None	Good quality, crystals at bottom
9	26.8	8.0	0.32	6.02	1.062	13.5	1.08	0.008	0.042		−3	Molds	Good quality
9a										−1	−3		Good quality
10	27.4	8.0	0.38	6.01	1.069	17.8	1.07	0.005	0.044		−4	None	Good quality
10a										+2	−2		Fair quality, crystals in milk
10b										+1	−2		Good quality, slight churning
10c										−1	−4		Good quality, crystals at bottom
10d										+1	−2		Good quality, slight churning
10e										+1	−2		Good quality, slight churning
10f										−1	−3		Good quality, slight churning

11	26.9	6.5	0.34	6.01	1.060	16.0	1.01	0.006	0.043		−4	None	Flavor scorched, crystals, dirty color
12	27.6	7.6	0.36	6.01	1.065	9.5	1.13	0.008	0.044		−4	None	Pungent flavor, slight churning
13	28.0	7.8	0.38	6.03	1.067	8.9	0.97	0.005	0.044		−2	820	Good quality
14	26.9	7.8	0.47	6.08	1.068	10.1	1.14	0.005	0.045		−3	None	Good quality
15	26.3	8.1	0.43	6.03	1.066	13.0	1.03	0.005	0.042		−2	None	Creamy flavor, light colored
16	27.0	7.6	0.43	6.03	1.067	11.7	1.11	0.005	0.046		−2	None	Bitter flavor, burned milk odor, churned
16a										+1	−3		Good quality
17	25.9	8.0	0.42	6.05	1.065	24.9	1.04	0.005	0.024		−3	None	Good quality, creamy
17a										−1	−2		Good quality
17b										−1	−3		Good quality
18	26.9	8.0	0.29	6.02	1.065	12.7	1.10	0.009	0.053		−3	None	Good quality, slight churning
19	25.8	7.8	0.30	6.04	1.064	9.1	1.00	0.004	0.042		−3	None	Good quality
19a										+2	−1		Good quality, crystals at bottom
20	26.4	7.8	0.34	6.04	1.066	9.7	1.10	0.010	0.040		−4	None	Poor quality, scorched, churned, pungent flavor
21	26.3	8.0	0.30	6.03	1.065	16.0	1.11	0.009	0.046		−3	None	Good quality, crystals at bottom
21a										+2	−2		Fair flavor, slightly churned
21b										−1	−3		Flavor flat, slight churning
21c										+2	−1		Good quality
21d										−1	−3		Good quality
21e										+1	−3		Good quality
21f										+2	−2		Good quality, crystals
21g										+1	−2		Good quality, crystals
22	25.6	8.0	0.34	6.01	1.063	19.1	1.11	0.007	0.041		−3	None	Creamy, dark color slightly churned
23	24.9	8.2	0.34	5.95	1.067	40.0	1.03	0.009	0.042		−2	2,500	Slight malted flavor, crystals
23a										+1	−3		Good flavor, scorched milk odor
23b										−1	−3		Good quality
23c										−1	−3		Pungent flavor
24										−1	−3		Good quality
25										+2	−3		Good quality, slight churning

SUMMARY

Feathering of evaporated milk in hot coffee is promoted by: (*a*) Characteristics of the milk, especially the salt balance; (*b*) coffee which is concentrated; (*c*) prolonged extraction of the coffee grounds; (*d*) the use of small quantities of milk in the coffee; (*e*) the slow, rather than fast, addition of milk to the coffee.

An imperical feathering test was developed.

Examination of 52 cans of commercial evaporated milk indicated that feathering in hot coffee is not a common defect of the product now offered to the public.

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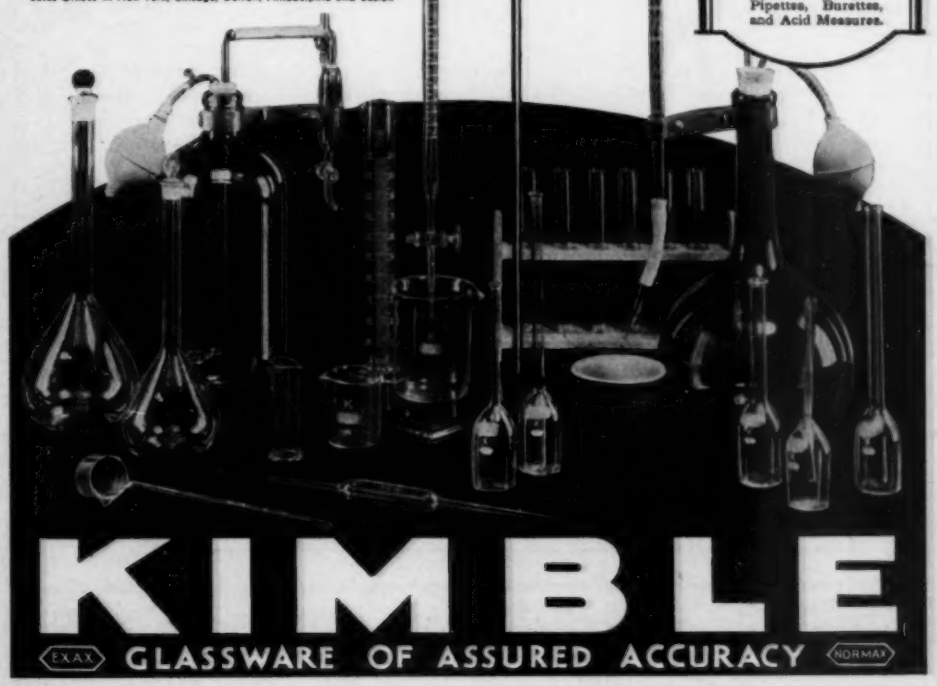

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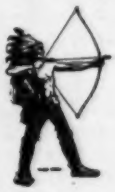

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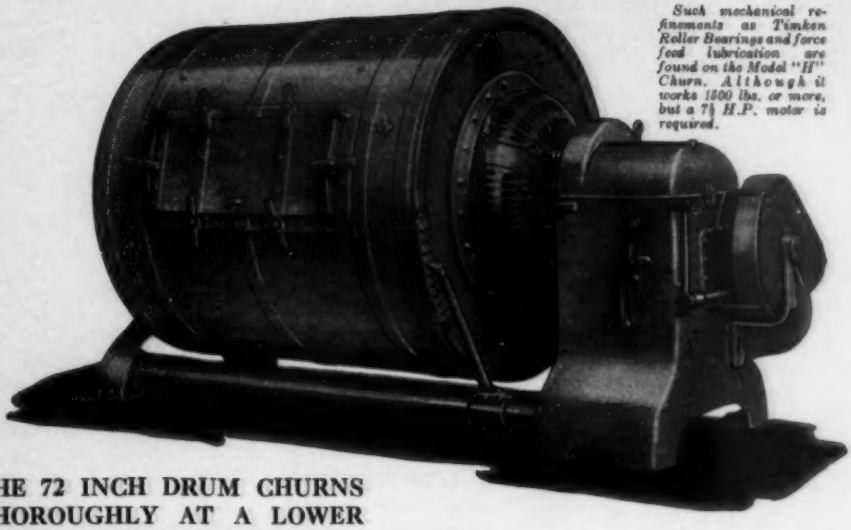

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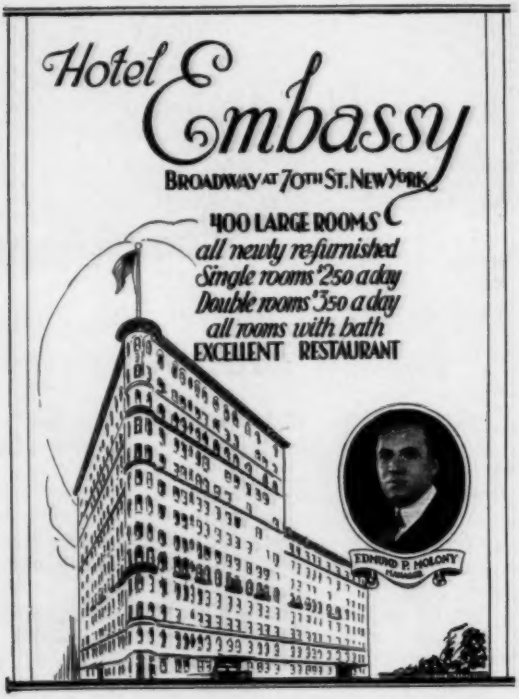

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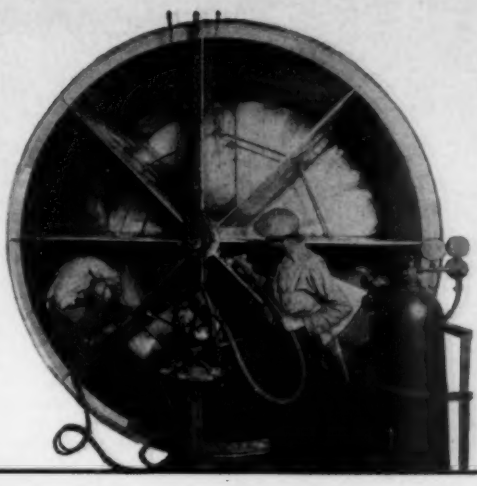

Building a "Mortensen" Nickel-lined positive holder at the Louisville, Kentucky, plant of the Standard Milk Machinery Company, Inc.

After the sheets are all riveted and sweated through, the Nickel alloy cover rim and heater seal is bolted and sweated in place. Then the Nickel alloy braces with flanges 12 inches in diameter are put in place, securely riveted and sweated to the partition sheets and soldered to a sanitary flush surface. The workman on the left is using a torch in building up the seams to a 1½ inch sanitary radius. The workman on the right is grinding the seams with a brush assuring a perfectly smooth, sanitary surface. This grinding cannot be done with plated or other surface coated metal.

TRADE MARK

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...positive holders protect dairy products better

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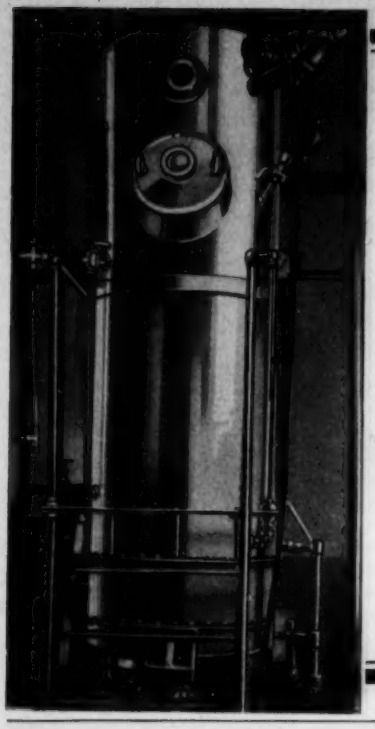

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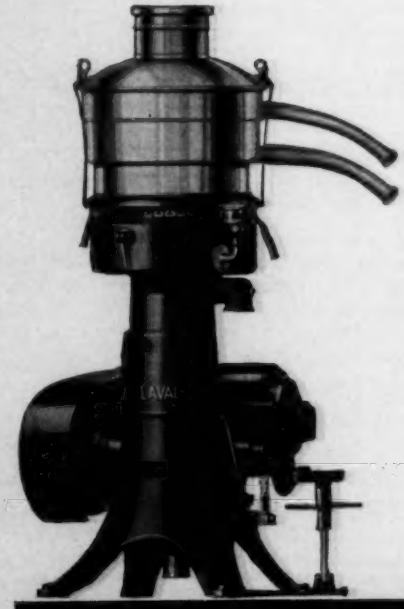

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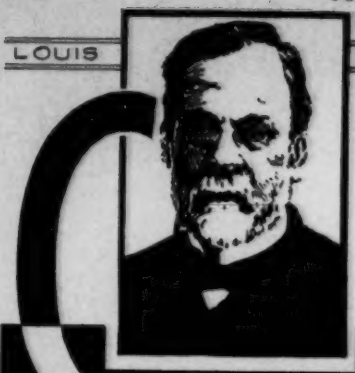

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